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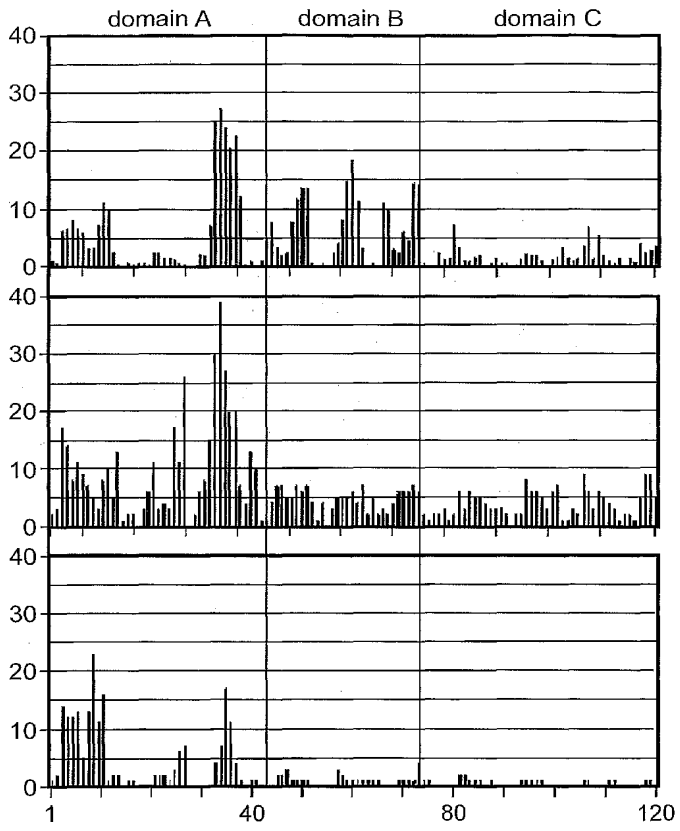
(54) Title: DOMAINS AND EPITOPES OF MENINGOCOCCAL PROTEIN NMB1870



(57) Abstract: 'NMB1870' is a known surface protein in *Neisseria meningitidis* expressed across all serogroups. It has three distinct families. Serum raised against a given family is bactericidal within the same family, but is not active against strains which express one of the other two families *i.e.* intra-family but not inter-family cross-protection. The inventors have found that NMB1870 can be divided into domains, and that not all domains are required for antigenicity. Antigenic domains can be taken from each of the three NMB1870 families and expressed as a single polypeptide chain. The inventors have also found that NMB1870 exposes some of its epitopes in surface loops situated between alpha helices, and that substitution of loop epitopes from one family into the loop position in another family allows chimeric NMB1870 to be produced with multi-family antigenicity. Thus chimeric NMB1870 proteins are provided that comprise portions of NMB1870 from different families.

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DOMAINS AND EPITOPES OF MENINGOCOCCAL PROTEIN NMB1870

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the field of immunisation and, in particular, immunisation against diseases caused by pathogenic bacteria in the genus *Neisseria*, such as *N.meningitidis* (meningococcus).

BACKGROUND ART

Neisseria meningitidis is a Gram-negative encapsulated bacterium which colonises the upper respiratory tract of approximately 10% of human population. Although polysaccharide and conjugate vaccines are available against serogroups A, C, W135 and Y, this approach cannot be applied to serogroup B because the capsular polysaccharide is a polymer of polysialic acid, which is a self antigen in humans. To develop a vaccine against serogroup B, surface-exposed proteins contained in outer membrane vesicles (OMVs) have been used. These vaccines elicit serum bactericidal antibody responses and protect against disease, but they fail to induce cross-strain protection [1]. Some workers are therefore focusing on specific meningococcal antigens for use in vaccines.

One such antigen is 'NMB1870'. This protein was originally disclosed as protein '741' from strain MC58 [SEQ IDs 2535 & 2536 in ref. 2; SEQ ID 1 herein], and has also been referred to as 'GNA1870' [ref. 3, following ref. 4] and as 'ORF2086' [5,6]. This protein is expressed across all meningococcal serogroups and has been found in multiple meningococcal strains. NMB1870 sequences group into three families, and it has been found that serum raised against a given family is bactericidal within the same family, but is not active against strains which express one of the other two families *i.e.* there is intra-family cross-protection, but not inter-family cross-protection.

To achieve cross-strain protection using NMB1870, therefore, more than one family is used. To avoid the need to express and purify separate proteins, it has been proposed to express different families as hybrid proteins [7], including two or three of the families in a single polypeptide chain. Several hybrids have been tested and give encouraging anti-meningococcal efficacy.

It is an object of the invention to provide further and improved approaches for overcoming the family specificity of protection afforded by NMB1870, and to use these approaches for providing immunity against meningococcal disease and/or infection, particularly for serogroup B.

DISCLOSURE OF THE INVENTION

The inventors have found that NMB1870 can be divided into domains, and that not all domains are required for antigenicity. Antigenic domains can be taken from each of the three NMB1870 families and expressed as a single polypeptide chain. This approach is simpler than expressing complete NMB1870 sequences end-to-end in a single polypeptide chain.

The inventors have also found that NMB1870 exposes some of its epitopes in surface loops situated between alpha helices. Substitution of loop epitopes from one family into the loop position in another family allows chimeric NMB1870 to be produced with multi-family antigenicity.

Thus the invention provides chimeric NMB1870 proteins that comprise portions of NMB1870 from different families. Whereas each NMB1870 family can elicit antibodies (*e.g.* in mice) that are effective only against strains in the same NMB1870 family, chimeric polypeptides of the invention can elicit antibodies that recognise NMB1870 proteins from more than one family.

Bactericidal antibody responses are conveniently measured in mice and are a standard indicator of vaccine efficacy [*e.g.* see end-note 14 of reference 4]. Chimeric proteins can preferably elicit an antibody response which is bactericidal against at least one *N.meningitidis* strain from each of at least two of the following three groups of strains:

(I) MC58, gb185 (=M01-240185), m4030, m2197, m2937, iss1001, NZ394/98, 67/00, 93/114, bz198, m1390, nge28, lnp17592, 00-241341, f6124, 205900, m198/172, bz133, gb149 (=M01-240149), nm008, nm092, 30/00, 39/99, 72/00, 95330, bz169, bz83, cu385, h44/76, m1590, m2934, m2969, m3370, m4215, m4318, n44/89, 14847.

(II) 961-5945, 2996, 96217, 312294, 11327, a22, gb013 (=M01-240013), e32, m1090, m4287, 860800, 599, 95N477, 90-18311, c11, m986, m2671, 1000, m1096, m3279, bz232, dk353, m3697, ngh38, L93/4286.

(III) M1239, 16889, gb355 (=M01-240355), m3369, m3813, ngp165.

For example, a chimeric polypeptide can elicit a bactericidal response effective against two or more of serogroup B *N.meningitidis* strains MC58, 961-5945 and M1239.

The chimeric polypeptide can preferably elicit an antibody response which is bactericidal against at least 50% of clinically-relevant meningococcal serogroup B strains (*e.g.* 60%, 70%, 80%, 90%, 95% or more). The chimeric polypeptide may elicit an antibody response which is bactericidal against strains of serogroup B *N.meningitidis* and strains of at least one (*e.g.* 1, 2, 3, 4) of serogroups A, C, W135 and Y. The chimeric polypeptide may elicit an antibody response which is bactericidal against strains of *N.gonococcus* and/or *N.cinerea*. The chimeric polypeptide may elicit an antibody response which is bactericidal against strains from at least two of the three main branches of the dendrogram shown in Figure 5 of reference 3.

The chimeric polypeptide may elicit an antibody response which is bactericidal against *N.meningitidis* strains in at least 2 (*e.g.* 2, 3, 4, 5, 6, 7) of hypervirulent lineages ET-37, ET-5, cluster A4, lineage 3, subgroup I, subgroup III, and subgroup IV-1 [8,9]. Chimeras may additionally induce bactericidal antibody responses against one or more hyperinvasive lineages.

Chimeras may elicit an antibody response which is bactericidal against *N.meningitidis* strains in at least at least 2 (*e.g.* 2, 3, 4, 5, 6, 7) of the following multilocus sequence types: ST1, ST4, ST5, ST8, ST11, ST32 and ST41 [10]. The chimera may also elicit an antibody response which is bactericidal against ST44 strains.

The composition need not induce bactericidal antibodies against each and every MenB strain within the specified lineages or MLST; rather, for any given group of four of more strains of serogroup B meningococcus within a particular hypervirulent lineage or MLST, the antibodies induced by the composition are bactericidal against at least 50% (*e.g.* 60%, 70%, 80%, 90% or more) of the group.

Preferred groups of strains will include strains isolated in at least four of the following countries: GB, AU, CA, NO, IT, US, NZ, NL, BR, and CU. The serum preferably has a bactericidal titre of at least 1024 (*e.g.* 2^{10}, 2^{11}, 2^{12}, 2^{13}, 2^{14}, 2^{15}, 2^{16}, 2^{17}, 2^{18} or higher, preferably at least 2^{14}) *i.e.* the serum is able to kill at least 50% of test bacteria of a particular strain when diluted 1:1024 *e.g.* as described in end-note 14 of reference 4. Preferred chimeric polypeptides can elicit an antibody response in mice that remains bactericidal even when the serum is diluted 1:4096 or further.

NMB1870 domains

SEQ ID NO: 1 is the full-length family I NMB1870 sequence from serogroup B strain MC58:

MNRTAFCCLSLTTALILTACSSGGGGVAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKLK
LAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTAFQTEQ
IQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFAAKQGNG
KIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGSAEVKTVNG
IRHIGLAAKQ

The N-terminus of the mature processed lipoprotein is underlined (Cys-20). The full-length sequence has been split into three domains (aa. 1-119, 120-183 and 184-274):

MNRTAFCCLSLTTALILTACSSGGGGVAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKLK
LAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYK**QSHSALTAFQTEQ**
IQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFAAKQGNG
KIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGSAEVKTVNG
IRHIGLAAKQ

From N-terminus to C-terminus these three domains are called 'A', 'B' and 'C'. The mature form of domain 'A', from the mature C-terminus cysteine, is called 'A_{mature}'.

For MC58, the domains are: 'A' = SEQ ID NO: 4; 'B' = SEQ ID NO: 5; 'C' = SEQ ID NO: 6; and 'A_{mature}' = SEQ ID NO: 13. Multiple NMB1870 sequences are known [*e.g.* see refs. 3, 6 and 7] and can readily be aligned using standard methods. By such alignments the skilled person can identify domains 'A' (and 'A_{mature}'), 'B' and 'C' in any given NMB1870 sequence by comparison to the coordinates in the MC58 sequence. For ease of reference, however, the domains are defined below:

- Domain 'A' in a given NMB1870 sequence is the fragment of that sequence which, when aligned to SEQ ID NO: 1 using a pairwise alignment algorithm, starts with the amino acid aligned to Met-1 of SEQ ID NO: 1 and ends with the amino acid aligned to Lys-119 of SEQ ID NO: 1.
- Domain 'A_{mature}' in a given NMB1870 sequence is the fragment of that sequence which, when aligned to SEQ ID NO: 1 using a pairwise alignment algorithm, starts with the amino acid aligned to Cys-20 of SEQ ID NO: 1 and ends with the amino acid aligned to Lys-119 of SEQ ID NO: 1.
- Domain 'B' in a given NMB1870 sequence is the fragment of that sequence which, when aligned to SEQ ID NO: 1 using a pairwise alignment algorithm, starts with the amino acid aligned to Gln-120 of SEQ ID NO: 1 and ends with the amino acid aligned to Gly-183 of SEQ ID NO: 1.

– Domain 'C' in a given NMB1870 sequence is the fragment of that sequence which, when aligned to SEQ ID NO: 1 using a pairwise alignment algorithm, starts with the amino acid aligned to Lys-184 of SEQ ID NO: 1 and ends with the amino acid aligned to Gln-274 of SEQ ID NO: 1.

The preferred pairwise alignment algorithm for defining the domains is the Needleman-Wunsch global alignment algorithm [11], using default parameters (*e.g.* with Gap opening penalty = 10.0, and with Gap extension penalty = 0.5, using the EBLOSUM62 scoring matrix). This algorithm is conveniently implemented in the *needle* tool in the EMBOSS package [12].

NMB1870 sequences fall into three families [3,7] that are referred to herein as families I, II and III. The prototypic sequences for families I-III are, respectively, SEQ ID NOS: 1-3. The phylogenetic and dendrogram methods of reference 3 can be followed in order to readily determine the family for any given NMB1870 sequence, and a pairwise alignment with each of the three prototypic NMB1870 sequences can also be used to find the closest family match. Sequences fall distinctly into the three families, with sequence identity being 74.1% between families I & II, 62.8% between families I & III and 84.7% between families II & III, and with sequence variation within each family being low (*e.g.* a minimum of 91.6% identity in family I, 93.4% in family II and 93.2% in family III). As a quick way of determining a sequence's family without requiring a phylogenetic analysis, a sequence can be placed in family I if it has at least 85% sequence identity to SEQ ID NO: 1, can be placed in family II if it has at least 85% sequence identity to SEQ ID NO: 2, and can be placed in family III if it has at least 85% sequence identity to SEQ ID NO: 3.

Based on the alignment in Figure 6 of reference 3, exemplary domains A, B and C for the three prototypic families of NMB1870 (SEQ ID NOS: 1 to 3) are as follows:

Family / Domain	A	B	C
I	SEQ ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 6
II	SEQ ID NO: 7	SEQ ID NO: 8	SEQ ID NO: 9
III	SEQ ID NO: 10	SEQ ID NO: 11	SEQ ID NO: 12

Preferred domains for use with the invention comprise amino acid sequences that (a) have at least $x\%$ sequence identity to one or more of SEQ ID NOS: 4 to 12, and/or (a) comprise a fragment of at least y consecutive amino acids sequence from one or more of SEQ ID NOS: 4 to 12.

The value of x is selected from 50, 60, 70, 75, 80, 85, 90, 92, 94, 95, 96, 97, 98, 99, 99.5, 99.9 or more. The value of y is selected from 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, 30, 35, 40, 45, 50 or more. In polypeptides comprising NMB1870 sequences from different families, the values of x and y for each family can be the same or different.

A domain 'A' sequence is preferably between a_1 and a_2 (inclusive) amino acids long, where: a_1 is selected from 110, 115, 120, 125 and 130; and a_2 is selected from 115, 120, 125, 130 and 135.

A domain 'B' sequence is preferably between b_1 and b_2 (inclusive) amino acids long, where: b_1 is selected from 55, 60, 65 and 70; and b_2 is selected from 60, 65, 70 and 75.

A domain 'C' sequence is preferably between c_1 and c_2 (inclusive) amino acids long, where: c_1 is selected from 80, 85, 90, 95 and 100; and c_2 is selected from 85, 90, 95, 100 and 105.

NMB1870 surface loops

The surface loops of SEQ ID NO: 1, lying between alpha helices, are: (1) amino acids 134-141; (2) amino acids 162-168; (3) amino acids 181-182; (4) amino acid 197; (5) amino acids 219-223; (6) amino acids 234-236; (7) amino acids 261-267:

MNRTAFCCLSLTTALILTACSSGGGGVAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKLK
LAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTAFQTEQ
IQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFAAKQGNG
KIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGSAEVKTVNG
IRHIGLAAKQ

By aligning SEQ ID NO: 1 with any other NMB1870 sequence, the skilled person can identify the positions of loops (1) to (7) in that sequence. For ease of reference, however, the coordinates of a loop are defined herein as the string of amino acid(s) in a NMB1870 sequence that, when aligned to SEQ ID NO: 1 using a pairwise alignment algorithm, starts with the amino acid aligned to the first amino acid residue of the loop defined above in SEQ ID NO: 1 above and ends with the last amino acid of the loop defined above in SEQ ID NO: 1.

Chimeric proteins

Joining heterologous domains B and C

The invention provides a chimeric polypeptide comprising: (a) a domain 'B' sequence from a first NMB1870 family; and (b) a domain 'C' sequence from a second NMB1870 family. The first and second family are each selected from I, II or III, but are not the same as each other. The chimeric polypeptide preferably does not contain a domain 'C' sequence from the first NMB1870 family and/or does not contain a domain 'B' sequence from the second NMB1870 family. The chimeric polypeptide is preferably less than 495 amino acids long.

Preferred polypeptides comprise an amino acid sequence $-X_1-B-X_2-C-X_3-$, wherein: $-X_1-$ is an optional amino acid sequence; $-X_2-$ is an optional amino acid sequence; $-X_3-$ is an optional amino acid sequence; -B- is a domain B amino acid sequence from a NMB1870 sequence in a first family; and -C- is a domain C amino acid sequence from a NMB1870 sequence in a second family. The -B- domain to the C-terminus of the -C- domain, but is preferably to the N-terminus of the -C- domain.

Preferably: (1) the domain B sequence of the first NMB1870 family (i) has at least *x%* sequence identity to SEQ ID NO: 5, SEQ ID NO: 8 or SEQ ID NO: 11, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: 5, SEQ ID NO: 8 or SEQ ID NO: 11; and (2) the domain C sequence of the second NMB1870 family (i) has at least *x%* sequence identity to SEQ ID NO: 6, SEQ ID NO: 9 or SEQ ID NO: 12, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: 6, SEQ ID NO: 9 or SEQ ID NO: 12; provided that the two SEQ ID NOS chosen for (1) and (2) are not (i) 5 and 6 together, (2) 8 and 9 together or

(3) 11 and 12 together. Suitable pairs of SEQ ID NOS to be combined for (1) and (2) are thus 5 & 9, 5 & 12, 8 & 6, 8 & 12, 11 & 6 and 11 & 9.

Joining heterologous BC domains

The invention provides a chimeric polypeptide comprising: (a) a domain 'B' sequence and a domain 'C' sequence from a first family of NMB1870; and (b) a domain 'B' sequence and a domain 'C' sequence from a second family of NMB1870. The chimeric polypeptide preferably does not contain a domain 'A' sequence from the first family and/or does not contain a domain 'A' sequence from the second family. The first and second family are each selected from I, II or III, but are not the same as each other.

The domain 'B' and 'C' sequences from the first family are preferably contiguous (a 'BC' domain). Similarly, the domain 'B' and 'C' sequences from the second family are preferably contiguous.

Preferred polypeptides comprise an amino acid sequence $-X_1-B_j-X_2-C_j-X_3-B_k-X_4-C_k-X_5-$, wherein: $-X_1-$ is an optional amino acid sequence; $-X_2-$ is an optional amino acid sequence; $-X_3-$ is an optional amino acid sequence; $-X_4-$ is an optional amino acid sequence; $-X_5-$ is an optional amino acid sequence; $-B_j-$ is a domain 'B' amino acid sequence from a first NMB1870 family; $-C_j-$ is a domain 'C' amino acid sequence from the first family; $-B_k-$ is a domain 'B' amino acid sequence from a second NMB1870 family; and $-C_k-$ is a domain 'C' amino acid sequence from the second family. Sequences $-X_2-$ and $-X_4-$ are preferably absent *i.e.* to give $-X_1-B_j-C_j-X_3-B_k-C_k-X_5-$.

It is preferred to have the $-B_j-$ and $-C_j-$ domains to the N-terminus of the $-B_k-$ and $-C_k-$ domains.

Preferably: (1) the domain B sequence of the first family (i) has at least *x%* sequence identity to SEQ ID NO: *J1*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *J1*; (2) the domain C sequence of the first family (i) has at least *x%* sequence identity to SEQ ID NO: *J2*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *J2*; (3) the domain B sequence of the second family (i) has at least *x%* sequence identity to SEQ ID NO: *K1*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *K1*; and (4) the domain C sequence of the second family (i) has at least *x%* sequence identity to SEQ ID NO: *K2*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *K2*, where *J1, J2, K1* and *K2* are selected as follows:

	J1	*J2*	*K1*	*K2*
(a)	5	6	8	9
(b)	5	6	11	12
(c)	8	9	5	6
(d)	8	9	11	12
(e)	11	12	5	6
(f)	11	12	8	9

The above polypeptides thus comprise BC domains from at least two of the three NMB1870 families. More preferably, the polypeptides comprise a BC domain from each of the three families. Thus the invention provides a chimeric polypeptide comprising: (a) a domain 'B' sequence and a domain 'C'

sequence from a first NMB1870 family; (b) a domain 'B' sequence and a domain 'C' sequence from a second NMB1870 family; and (c) a domain 'B' sequence and a domain 'C' sequence from a third NMB1870 family. The chimeric polypeptide preferably does not contain a domain 'A' sequence from the first family and/or does not contain a domain 'A' sequence from the second family and/or does not contain a domain 'A' sequence from the third family.

The domain 'B' and 'C' sequences from the first family are preferably contiguous. Similarly, the domain 'B' and 'C' sequences from the second family are preferably contiguous. Similarly, the domain 'B' and 'C' sequences from the third family are preferably contiguous.

Preferred polypeptides comprise an amino acid sequence $-X_1-B_j-X_2-C_j-X_3-B_k-X_4-C_k-X_5-B_L-X_6-C_L-X_7-$ wherein: $-X_1-$ is an optional amino acid sequence; $-X_2-$ is an optional amino acid sequence; $-X_3-$ is an optional amino acid sequence; $-X_4-$ is an optional amino acid sequence; $-X_5-$ is an optional amino acid sequence; $-X_6-$ is an optional amino acid sequence; $-X_7-$ is an optional amino acid sequence; $-B_j-$ is a domain B amino acid sequence from a first NMB1870 family; $-C_j-$ is a domain C amino acid sequence from the first family; $-B_k-$ is a domain B amino acid sequence from a second NMB1870 family; $-C_k-$ is a domain C amino acid sequence from the second family; $-B_L-$ is a domain B amino acid sequence from a third NMB1870 family; and $-C_L-$ is a domain C amino acid sequence from the third family. Sequences $-X_2-$, $-X_4-$ and $-X_6-$ are preferably absent *i.e.* to give $-X_1-B_j-C_j-X_3-B_k-C_k-X_5-B_l-C_l-X_7-$.

Preferably: (1) the domain B sequence of the first family (i) has at least *x%* sequence identity to SEQ ID NO: *J1*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *J1*; (2) the domain C sequence of the first family (i) has at least *x%* sequence identity to SEQ ID NO: *J2*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *J2*; (3) the domain B sequence of the second family (i) has at least *x%* sequence identity to SEQ ID NO: *K1*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *K1*; (4) the domain C sequence of the second family (i) has at least *x%* sequence identity to SEQ ID NO: *K2*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *K2*; (5) the domain B sequence of the third family (i) has at least *x%* sequence identity to SEQ ID NO: *L1*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *L1*; and (6) the domain C sequence of the third family (i) has at least *x%* sequence identity to SEQ ID NO: *L2*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *L2*, where *J1, J2, K1, K2, L1* and *L2* are selected as follows:

	J1	*J2*	*K1*	*K2*	*L1*	*L2*
(a)	5	6	8	9	11	12
(b)	5	6	11	12	8	9
(c)	8	9	5	6	11	12
(d)	8	9	11	12	5	6
(e)	11	12	5	6	8	9
(f)	11	12	8	9	5	6

Joining heterologous AB domains

The invention provides a chimeric polypeptide comprising: (a) a domain 'A' sequence and a domain 'B' sequence from a first family of NMB1870; and (b) a domain 'A' sequence and a domain 'B' sequence from a second family of NMB1870. The chimeric polypeptide preferably does not contain a domain 'C' sequence from the first family and/or does not contain a domain 'C' sequence from the second family. The first and second family are each selected from I, II or III, but are not the same as each other.

The domain 'A' and 'B' sequences from the first family are preferably contiguous. Similarly, the domain 'A' and 'B' sequences from the second family are preferably contiguous.

Preferred polypeptides comprise an amino acid sequence $-X_1-A_j-X_2-B_j-X_3-A_k-X_4-B_k-X_5-$, wherein: $-X_1-$ is an optional amino acid sequence; $-X_2-$ is an optional amino acid sequence; $-X_3-$ is an optional amino acid sequence; $-X_4-$ is an optional amino acid sequence; $-X_5-$ is an optional amino acid sequence; $-A_j-$ is a domain A amino acid sequence from a first NMB1870 family; $-B_j-$ is a domain B amino acid sequence from the first family; $-A_k-$ is a domain A amino acid sequence from a second NMB1870 family; and $-B_k-$ is a domain B amino acid sequence from the second family. Sequences $-X_2-$ and $-X_4-$ are preferably absent *i.e.* to give $-X_1-A_j-B_j-X_3-A_k-B_k-X_5-$.

It is preferred to have the $-A_j-$ and $-B_j-$ domains to the N-terminus of the $-A_k-$ and $-B_k-$ domains.

Preferably: (1) the domain A sequence of the first family (i) has at least *x%* sequence identity to SEQ ID NO: *J1*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *J1*; (2) the domain B sequence of the first family (i) has at least *x%* sequence identity to SEQ ID NO: *J2*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *J2*; (3) the domain A sequence of the second family (i) has at least *x%* sequence identity to SEQ ID NO: *K1*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *K1*; and (4) the domain B sequence of the second family (i) has at least *x%* sequence identity to SEQ ID NO: *K2*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *K2*, where *J1, J2, K1* and *K2* are selected as follows:

	J1	*J2*	*K1*	*K2*
(a)	4	5	7	8
(b)	4	5	10	11
(c)	7	8	4	5
(d)	7	8	10	11
(e)	10	11	4	5
(f)	10	11	7	8

The above polypeptides thus comprise AB domains from at least two of the three NMB1870 families. More preferably, the polypeptides comprise a AB domain from each of the three families. Thus the invention provides a chimeric polypeptide comprising: (a) a domain 'A' sequence and a domain 'B' sequence from a first family of NMB1870; (b) a domain 'A' sequence and a domain 'B' sequence from a second family of NMB1870; and (c) a domain 'A' sequence and a domain 'B'

sequence from a third family of NMB1870. The chimeric polypeptide preferably does not contain a domain 'C' sequence from the first family and/or does not contain a domain 'C' sequence from the second family and/or does not contain a domain 'C' sequence from the third family.

The domain 'A' and 'B' sequences from the first family are preferably contiguous. Similarly, the domain 'A' and 'B' sequences from the second family are preferably contiguous. Similarly, the domain 'A' and 'B' sequences from the third family are preferably contiguous.

Preferred polypeptides have an amino acid sequence $-X_1-A_j-X_2-B_j-X_3-A_k-X_4-B_k-X_5-A_L-X_6-B_L-X_7-$, wherein: $-X_1-$ is an optional amino acid sequence; $-X_2-$ is an optional amino acid sequence; $-X_3-$ is an optional amino acid sequence; $-X_4-$ is an optional amino acid sequence; $-X_5-$ is an optional amino acid sequence; $-X_6-$ is an optional amino acid sequence; $-X_7-$ is an optional amino acid sequence; $-A_j-$ is a domain A amino acid sequence from a first NMB1870 family; $-B_j-$ is a domain B amino acid sequence from the first family; $-A_k-$ is a domain A amino acid sequence from a second NMB1870 family; $-B_k-$ is a domain B amino acid sequence from the second family; $-A_l-$ is a domain A amino acid sequence from a third NMB1870 family; and $-B_l-$ is a domain B amino acid sequence from the third family. Sequences $-X_2-$, $-X_4-$ and $-X_6-$ are preferably absent *i.e.* to give $-X_1-A_j-B_j-X_3-A_k-B_k-X_5-A_l-B_l-X_7-$.

Preferably: (1) the domain A sequence of the first family (i) has at least *x%* sequence identity to SEQ ID NO: *J1*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *J1*; (2) the domain B sequence of the first family (i) has at least *x%* sequence identity to SEQ ID NO: *J2*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *J2*; (3) the domain A sequence of the second family (i) has at least *x%* sequence identity to SEQ ID NO: *K1*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *K1*; (4) the domain B sequence of the second family (i) has at least *x%* sequence identity to SEQ ID NO: *K2*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *K2*; (5) the domain A sequence of the third family (i) has at least *x%* sequence identity to SEQ ID NO: *L1*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *L1*; and (6) the domain B sequence of the third family (i) has at least *x%* sequence identity to SEQ ID NO: *L2*, and/or (ii) comprises a fragment of at least *y* consecutive amino acids sequence from SEQ ID NO: *L2*, where *J1, J2, K1, K2, L1* and *L2* are selected as follows:

	J1	*J2*	*K1*	*K2*	*L1*	*L2*
(a)	4	5	7	8	10	11
(b)	4	5	10	11	7	8
(c)	7	8	4	5	10	11
(d)	7	8	10	11	4	5
(e)	10	11	4	5	7	8
(f)	10	11	7	8	4	5

Optional X_n sequences

Polypeptides of the invention may include sequences linking NMB1870-derived sequences and/or may include N- and C-terminal sequences that are not derived from NMB1870. Such sequences are designated "X_n" herein (X_1, X_2, X_3, X_4, X_5, X_6, X_7, *etc.*). Each X_n may be present or absent, and the sequence of each may be the same or different.

Linker amino acid sequence(s) will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* Gly_n where n = 2, 3, 4, 5, 6, 7, 8, 9, 10 or more), and histidine tags (*i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG (SEQ ID NO: 17), with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and another useful linker is GKGGGG (SEQ ID NO: 45), with the Gly-Lys dipeptide being formed from a *Hin*dIII restriction site. The restriction sites are followed by the Gly_4 tetrapeptide (SEQ ID NO: 18), which is a typical poly-glycine linker. Other useful linkers are SEQ ID NOS: 19 and 20.

Optional N-terminal amino acid sequences will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct polypeptide trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be apparent to those skilled in the art. If a sequence lacks its own N-terminus methionine then a useful N-terminal sequence will provide such a methionine residue in the translated polypeptide (*e.g.* a single Met residue).

Optional C-terminal amino acid sequences will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct polypeptide trafficking, short peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more), or sequences which enhance polypeptide stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

Constructing chimeric NMB1870 sequences

The invention provides a process for producing a chimeric NMB1870 amino acid sequence, comprising the steps of: (a) aligning a first NMB1870 amino acid sequence with a second NMB1870 amino acid sequence, to give a pair of aligned sequences; (b) selecting a portion of the first amino acid sequence, starting at amino acid a_1 of said first amino acid sequence and ending at amino acid b_1 of said first amino acid sequence; (c) selecting a portion of the second amino acid sequence, starting at amino acid a_2 of said second amino acid sequence and ending at amino acid b_2 of said second amino acid sequence, wherein residues a_1 & a_2 and b_1 & b_2 are aligned in the pair of aligned sequences; and (d) replacing said portion of the first amino acid sequence with said portion of the

second amino acid sequence, thereby providing the chimeric NMB1870 amino acid sequence. The first and second sequences are different, and are preferably from different NMB1870 families.

Steps (b) to (d) may be performed more than once for the same alignment from step (a) *i.e.* multiple substitutions from the second sequence into the first sequence can be performed. Similarly, steps (a) to (d) may be performed more than once, with a different "second amino acid sequence" optionally being used during subsequent steps (a) *i.e.* a first sequence can be aligned with a second sequence and subjected to the substitution procedure, and then may be aligned with a different second sequence and subjected to a further substitution, *etc.*

Thus the invention provides a process for producing a chimeric NMB1870 amino acid sequence, comprising the steps of: (a) aligning a first NMB1870 amino acid sequence with a second NMB1870 amino acid sequence, to give a first pair of aligned sequences; (b) selecting a portion of the first amino acid sequence, starting at amino acid a_1 of said first amino acid sequence and ending at amino acid b_1 of said first amino acid sequence; (c) selecting a portion of the second amino acid sequence, starting at amino acid a_2 of said second amino acid sequence and ending at amino acid b_2 of said second amino acid sequence, wherein residues a_1 & a_2 and b_1 & b_2 are aligned in the first pair of aligned sequences; (d) replacing said portion of the first amino acid sequence with said portion of the second amino acid sequence, thereby providing an intermediate chimeric NMB1870 amino acid sequence; (e) aligning the first NMB1870 amino acid sequence, or the intermediate chimeric sequence, with a third NMB1870 amino acid sequence, to give a second pair of aligned sequences; (f) selecting a portion of the intermediate chimeric sequence, starting at amino acid a_3 of the intermediate chimeric sequence, and ending at amino acid b_3 of the intermediate chimeric sequence; (g) selecting a portion of the third amino acid sequence, starting at amino acid a_4 of said third amino acid sequence and ending at amino acid b_4 of said third amino acid sequence, wherein residues a_3 & a_4 and b_3 & b_4 are aligned in the second pair of aligned sequences; and (h) replacing said portion of the intermediate chimeric sequence, with said portion of the third amino acid sequence, thereby providing the chimeric NMB1870 amino acid sequence.

The selected portions are preferably at least c amino acids long, where c is 3, 4, 5, 6, 7, 8, 9 or more.

The substituted sequence(s) are preferably surface loop sequences. The invention includes situations including up to 10 substitutions (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) or more.

The invention also provides a polypeptide comprising a chimeric NMB1870 amino acid sequence, wherein said chimeric NMB1870 amino acid sequence is obtainable by the above process.

The process of the invention may be followed by the further step of producing a polypeptide comprising said chimeric NMB1870 amino acid sequence *e.g.* by recombinant protein expression.

The invention provides a polypeptide comprising an amino acid sequence F_1-X_1-F_2, where: F_1 is a N-terminus fragment of a first NMB1870 amino acid sequence; F_2 is a C-terminus fragment of a second NMB1870 amino acid sequence; -X_1- is an optional amino acid sequence; said first and second NMB1870 amino acid sequences are from different NMB1870 families; fragments F_1 and F_2

are both at least 10 amino acids in length; and fragments F_1 and F_2 have a combined length of at least *ff* amino acids. The value of *ff* is 200, 210, 220, 230, 240, 250 or 260. The $-X_1-$ sequence is preferably absent, to give sequence F_1-F_2, which is a fusion of the N- and C- termini from NMB1870 proteins in different families. The invention also provides a fragment of at least *g* consecutive amino acids of said polypeptide, provided that said fragment includes at least one amino acid from each of F_1 and F_2 (*i.e.* the fragment bridged the join between F_1 and F_2. The value of *g* is 7, 8, 9, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, 75, 100 or more.

The invention provides a polypeptide comprising an amino acid sequence $(F^m-X^m)_n$, where: each F^m is a fragment of a m^{th} NMB1870 amino acid sequence; each $-X^m-$ is an optional amino acid sequence; each fragment F_m is at least *g* amino acids in length; and the *n* instances of F_m include fragments from at least two of the three NMB1870 families I, II and III. The value of *g* is as defined above. The value of *n* is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

The invention provides a polypeptide comprising at least two of: (i) a fragment of no more than 240 amino acids of a family I NMB1870 sequence, wherein the fragment comprises an epitope of said family I NMB1870 sequence; (ii) a fragment of no more than 240 amino acids of a family II NMB1870 sequence, wherein the fragment comprises an epitope of said family II NMB1870 sequence; and (iii) a fragment of no more than 240 amino acids of a family III NMB1870 sequence, wherein the fragment comprises an epitope of said family III NMB1870 sequence.

Loop substitution

The inventors have found that NMB1870 exposes some of its epitopes in surface loops situated between alpha helices. Substitution of loop epitopes from one family into the loop position in another family allows chimeric NMB1870 to be produced with multi-family antigenicity.

Thus the invention provides a polypeptide comprising a modified amino acid sequence of a first family of NMB1870, wherein the modified sequence includes at least one (*e.g.* 1, 2, 3, 4, 5, 6 or 7) surface loop sequence from a second family of NMB1870 in place of a surface loop sequence from the first family.

The invention also provides a polypeptide comprising an amino acid sequence:

$$-B_1-L_1-B_2-L_2-B_3-L_3-B_4-L_4-B_5-L_5-B_6-L_6-B_7-L_7-B_8-$$

wherein: (a) each of said B_1, B_2, B_3, B_4, B_5, B_6, B_7 and B_8 is: (i) a fragment of SEQ ID NO: *M*; (ii) an amino acid sequence having at least *m%* sequence identity to said fragment of (i) and/or comprising a fragment of at least *mm* contiguous amino acids from said fragment of (i); (b) each of said L_1, L_2, L_3, L_4, L_5, L_6 and L_7 is: (iii) a fragment of SEQ ID NO: 1, SEQ ID NO: 2 and/or of SEQ ID NO: 3; (iv) an amino acid sequence having at least *n%* sequence identity to said fragment of (iii) and/or comprising a fragment of at least *nn* contiguous amino acids from said fragment of (iii), provided that at least one of said L_1, L_2, L_3, L_4, L_5, L_6 and L_7 is not a fragment of SEQ ID NO: *M*.

Thus the polypeptide comprises a basic backbone sequence, in eight parts, and seven loops, one between each consecutive part of backbone sequence, but at least one of the loop sequences is taken

from a NMB1870 sequence that is from a different NMB1870 family from the basic backbone sequence. It is preferred to use surface loops from more than one different NMB1870 sequences, and it is possible to insert these loops into a single backbone sequence.

The value of *M* is selected from 1, 2 or 3, and the definitions of B_1, B_2, B_3, B_4, B_5, B_6, B_7 and B_8 and of L_1, L_2, L_3, L_4, L_5, L_6 and L_7 vary depending on the value of *M*.

The meaning of "(i) a fragment of SEQ ID NO: *M*" is as follows:

	Amino acid co-ordinates within SEQ ID NO: *M*							
M	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8
1	1-133	142-161	169-180	183-196	198-218	224-233	237-260	268-274
2	1-133	142-161	168-179	182-195	197-217	223-232	236-259	267-273
3	1-141	150-169	176-187	190-203	205-225	231-240	244-267	275-281

Similarly, "(iii) a fragment of SEQ ID NO: 1, SEQ ID NO: 2 and/or of SEQ ID NO: 3" is defined as follows:

	Amino acid co-ordinates within SEQ ID NO: 1, 2 or 3						
SEQ	L_1	L_2	L_3	L_4	L_5	L_6	L_7
1	134-141	162-168	181-182	197	219-223	234-236	261-267
2	134-141	162-167	180-181	196	218-222	233-235	260-266
3	142-149	170-175	188-189	204	226-230	241-243	268-274

For example, the invention provides a polypeptide comprising an amino acid sequence:

$$-B_1-L_1-B_2-L_2-B_3-L_3-B_4-L_4-B_5-L_5-B_6-L_6-B_7-L_7-B_8-$$

wherein: B_1 is amino acids 1-139 of SEQ ID NO: 1, or an amino acid sequence having at least *m%* sequence identity to said amino acids 1-133 and/or comprising a fragment of at least *mm* contiguous amino acids from said amino acids 1-133; B_2 is amino acids 142-161 of SEQ ID NO: 1, or an amino acid sequence having at least *m%* sequence identity to said amino acids 142-161 and/or comprising a fragment of at least *mm* contiguous amino acids from said amino acids 142-161 ... B_7 is amino acids 268-274 of SEQ ID NO: 1, or an amino acid sequence having at least *m%* sequence identity to said amino acids 268-274 and/or comprising a fragment of at least *mm* contiguous amino acids from said amino acids 268-274; L_1 is amino acids 134-141 of SEQ ID NO: 2, or an amino acid sequence having at least *n%* sequence identity to said amino acids 134-141 and/or comprising a fragment of at least *nn* contiguous amino acids from said amino acids 134-141; L_2 is amino acids 162-167 of SEQ ID NO: 2, or an amino acid sequence having at least *n%* sequence identity to said amino acids 162-167 and/or comprising a fragment of at least *nn* contiguous amino acids from said amino acids 162-167, ... L_7 is amino acids 268-274 of SEQ ID NO: 3, or an amino acid sequence having at least *n%* sequence identity to said amino acids 268-274 and/or comprising a fragment of at least *nn* contiguous amino acids from said amino acids 268-274; *etc.*

The value of *m* is selected from 50, 60, 70, 75, 80, 85, 90, 92, 94, 95, 96, 97, 98, 99, 99.5, 99.9 or more. The value of *n* is selected from 50, 60, 70, 75, 80, 85, 90, 92, 94, 95, 96, 97, 98, 99, 99.5, 99.9

or more. The value of *mm* is selected from 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 40, 45, 50, 60, 70, 75, 100 or more. The value of *nn* is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. The value of *nn* is preferably less than 20.

The invention also provides a polypeptide comprising the chimeric amino acid sequence:

$$-B_1-L_1-B_2-L_2-B_3-L_3-B_4-L_4-B_5-L_5-B_6-L_6-B_7-L_7-B_8-$$

as defined above, and further comprising, either N-terminal to or C-terminal to said chimeric sequence, a NMB1870 sequence, wherein said NMB1870 sequence is in the same NMB1870 family as SEQ ID NO: *M*. Thus the polypeptide comprises both (i) a NMB1870 from a particular family and (ii) also a NMB1870 from the same family, but with at least one of its surface loops substituted for a different NMB1870 family.

The invention provides a polypeptide comprising an amino acid sequence that has an overall sequence identity to SEQ ID NO: *Q* of *q%*, wherein: the value of *q* is at least *r*; the sequence identity of said amino acid sequence to SEQ ID NO: *Q* is more than *q%* at the backbone regions of SEQ ID NO: *Q*; and the sequence identity of said amino acid sequence to SEQ ID NO: *Q* is less than *q%* at the loop regions of SEQ ID NO: *Q*. The value of *r* is selected from 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, and 99.5.

The value of *Q* is 1, 2 or 3, and the boundaries of the loop regions and of the backbone regions are selected accordingly from the above tables (L_1 to L_7 being the loops, and B_1 to B_8 being the backbone).

Where *Q* is 1, the amino acid sequence in a loop region may have more than *q%* sequence identity to the corresponding loop region of SEQ ID NO: 2 or SEQ ID NO: 3. Where *Q* is 2, the amino acid sequence in a loop region may have more than *q%* sequence identity to the corresponding loop region of SEQ ID NO: 1 or SEQ ID NO: 3. Where *Q* is 3, the amino acid sequence in a loop region may have more than *q%* sequence identity to the corresponding loop region of SEQ ID NO: 1 or SEQ ID NO: 2.

NMB1870 fragments

The invention provides a polypeptide comprising a fragment of a family I NMB1870 sequence, provided that (a) said fragment includes amino acid Arg-223 (b) said polypeptide comprises neither (i) a complete family I NMB1870 amino acid sequence nor (ii) a complete family I ? G-NMB1870 amino acid sequence. Numbering of amino acid residues follows the number of SEQ ID NO: 1 herein. The fragment may include complete domains B and C.

If said polypeptide includes an amino acid to the N-terminus of said fragment, then said amino acid immediately to the N-terminus of said fragment in said polypeptide is preferably different from the amino acid that is found immediately to the N-terminus of said fragment in SEQ ID NO: 1.

Similarly, if said polypeptide includes an amino acid to the C-terminus of said fragment, then said amino acid immediately to the C-terminus of said fragment in said polypeptide is preferably different from the amino acid that is found immediately to the C-terminus of said fragment in SEQ ID NO: 1.

The invention also provides a polypeptide comprising amino acid sequence -Z_1-Arg-Z_2-, wherein:

(a) -Z_1- is an amino acid sequence consisting of y_1 amino acids, wherein the value of y_1 is at least 10 (*e.g.* 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220 or more).

(b) -Z_2- is an amino acid sequence consisting of y_2 amino acids, wherein the value of y_2 is at least 10 (*e.g.* 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, 30, 35, 40, 45, 50 or more).

(c) -Z_1- has at least $x\%$ sequence identity (as defined above) to the y_1 amino acids located immediately upstream of amino acid Arg-223 in SEQ ID NO: 1; and

(d) –Z_2- has at least $x\%$ sequence identity (as defined above) to the y_2 amino acids located immediately downstream of amino acid Arg-223 in SEQ ID NO: 1.

The value of y_1 is preferably less than 220. The value of y_2 is preferably less than 50.

If the polypeptide includes an amino acid sequence upstream of -Z_1- then said sequence is preferably different from the sequence that is found immediately upstream of the y_1 amino acids located immediately upstream of amino acid Arg-223 in SEQ ID NO: 1.

If the polypeptide includes an amino acid sequence downstream of -Z_2- then said sequence is preferably different from the sequence that is found immediately downstream of the y_2 amino acids located immediately downstream of amino acid Arg-223 in SEQ ID NO: 1.

These fragment-including polypeptides of the invention preferably do not include any known polypeptides *e.g.* disclosed in references 2, 3, 6, 7, *etc.*

The invention also provides a mixture comprising a first polypeptide and a second polypeptide, where the first polypeptide is a fragment from domain B of a NMB1870 and the second polypeptide is a fragment from domain C of a NMB1870, wherein the first and second polypeptides can associate to produce an epitope that is not found on either the first or second polypeptide alone. For any given NMB1870 it is straightforward to identify domains B and C using the information supplied herein, and truncation and/or dissection of the separate domains, followed by mixing, can be used to see if the polypeptides associate. A convenient assay for determining association and formation of the conformational epitope involves the use of a monoclonal antibody that is recognises a domain BC fragment of NMB1870 but does not recognise domain B or C alone. Such antibodies can be isolated from polyclonal mouse anti-NMB1870 antiserum by standard screening methods.

Polypeptides

The invention provides the polypeptides described above.

It also provides a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 23, 24, 43, 44, 52, 53, 61, 62, 63, 64 and 65. It also provides polypeptides having an amino acid sequence (a) having sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 23, 24, 43, 44, 52, 53, 61, 62, 63, 64 and 65 and/or (b) comprising a fragment of an amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 23, 24, 43, 44, 52, 53, 61, 62, 63, 64 and 65. The degree of sequence identity is preferably greater than 50% (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more). The fragment preferably comprises 7 or more consecutive amino acids from the starting sequence (*e.g.* 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 45, 50, 55, 60, 65, 70, 75, 70, 85, 90, 95, 100 or more).

NMB1870 is naturally a lipoprotein in *N.meningitidis*. It has also been found to be lipidated when expressed in *E.coli*. Preferred polypeptides of the invention have a C-terminus cysteine residue, which may be lipidated *e.g.* comprising a palmitoyl group.

A characteristic of preferred polypeptides of the invention is the ability to induce bactericidal anti-meningococcal antibodies after administration to a host animal.

Polypeptides of the invention can be prepared by various means *e.g.* by chemical synthesis (at least in part), by digesting longer polypeptides using proteases, by translation from RNA, by purification from cell culture (*e.g.* from recombinant expression or from *N.meningitidis* culture). *etc.* Heterologous expression in an *E.coli* host is a preferred expression route (*e.g.* in DH5a, BL21(DE_3), BLR, *etc.*).

Polypeptides of the invention may be attached or immobilised to a solid support.

Polypeptides of the invention may comprise a detectable label *e.g.* a radioactive label, a fluorescent label, or a biotin label. This is particularly useful in immunoassay techniques.

Polypeptides can take various forms (*e.g.* native, fusions, glycosylated, non-glycosylated, lipidated, disulfide bridges, *etc.*).

Polypeptides are preferably prepared in substantially pure or substantially isolated form (*i.e.* substantially free from other Neisserial or host cell polypeptides) or substantially isolated form. In general, the polypeptides are provided in a non-naturally occurring environment *e.g.* they are separated from their naturally-occurring environment. In certain embodiments, the subject polypeptide is present in a composition that is enriched for the polypeptide as compared to a control. As such, purified polypeptide is provided, whereby purified is meant that the polypeptide is present in a composition that is substantially free of other expressed polypeptides, where by substantially free is meant that less than 90%, usually less than 60% and more usually less than 50% of the composition is made up of other expressed polypeptides.

The term "polypeptide" refers to amino acid polymers of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for

example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, *etc.*), as well as other modifications known in the art. Polypeptides can occur as single chains or associated chains.

Nucleic acids

The invention provides nucleic acid encoding a polypeptide of the invention as defined above. The invention also provides nucleic acid comprising: (a) a fragment of at least *n* consecutive nucleotides from said nucleic acid, wherein *n* is 10 or more (*e.g.* 12, 14, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500 or more); and/or (b) a sequence having at least 50% (*e.g.* 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more) sequence identity to said nucleic acid.

Furthermore, the invention provides nucleic acid which can chimericise to nucleic acid encoding a polypeptide of the invention, preferably under "high stringency" conditions (*e.g.* 65°C in a 0.1xSSC, 0.5% SDS solution).

Nucleic acids of the invention can be used in hybridisation reactions (*e.g.* Northern or Southern blots, or in nucleic acid microarrays or 'gene chips') and amplification reactions (*e.g.* PCR, SDA, SSSR, LCR, TMA, NASBA, *etc.*) and other nucleic acid techniques.

Nucleic acids of the invention may be prepared in many ways *e.g.* by chemical synthesis (*e.g.* phosphoramidite synthesis of DNA) in whole or in part, by digesting longer nucleic acids using nucleases (*e.g.* restriction enzymes), by joining shorter nucleic acids or nucleotides (*e.g.* using ligases or polymerases), from genomic or cDNA libraries, *etc.*

Nucleic acids of the invention can take various forms *e.g.* single-stranded, double-stranded, vectors, primers, probes, labelled, unlabelled, *etc.*

Nucleic acids of the invention are preferably in isolated or substantially isolated form.

The invention includes nucleic acid comprising sequences complementary to those described above *e.g.* for antisense or probing, or for use as primers.

The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA), *etc.*

Nucleic acid according to the invention may be labelled *e.g.* with a radioactive or fluorescent label. This is particularly useful where the nucleic acid is to be used in nucleic acid detection techniques *e.g.* where the nucleic acid is a primer or as a probe for use in techniques such as PCR, LCR, TMA, NASBA, *etc.*

The invention also provides vectors comprising nucleotide sequences of the invention (*e.g.* cloning or expression vectors, such as those suitable for nucleic acid immunisation) and host cells transformed with such vectors.

Immunisation

Polypeptides of the invention are preferably provided as immunogenic compositions, and the invention provides an immunogenic composition of the invention for use as a medicament.

The invention also provides a method for raising an antibody response in a mammal, comprising administering an immunogenic composition of the invention to the mammal. The antibody response is preferably a protective and/or bactericidal antibody response.

The invention also provides a method for protecting a mammal against a Neisserial (*e.g.* meningococcal) infection, comprising administering to the mammal an immunogenic composition of the invention.

The invention provides chimeric polypeptides of the invention for use as medicaments (*e.g.* as immunogenic compositions or as vaccines) or as diagnostic reagents. It also provides the use of nucleic acid, polypeptide, or antibody of the invention in the manufacture of a medicament for preventing Neisserial (*e.g.* meningococcal) infection in a mammal.

The mammal is preferably a human. The human may be an adult or, preferably, a child. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant); where the vaccine is for therapeutic use, the human is preferably an adult. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

The uses and methods are particularly useful for preventing/treating diseases including, but not limited to, meningitis (particularly bacterial meningitis) and bacteremia.

Efficacy of therapeutic treatment can be tested by monitoring Neisserial infection after administration of the composition of the invention. Efficacy of prophylactic treatment can be tested by monitoring immune responses against NMB1870 after administration of the composition. Immunogenicity of compositions of the invention can be determined by administering them to test subjects (*e.g.* children 12-16 months age, or animal models [13]) and then determining standard parameters including serum bactericidal antibodies (SBA) and ELISA titres (GMT). These immune responses will generally be determined around 4 weeks after administration of the composition, and compared to values determined before administration of the composition. A SBA increase of at least 4-fold or 8-fold is preferred. Where more than one dose of the composition is administered, more than one post-administration determination may be made.

Preferred compositions of the invention can confer an antibody titre in a patient that is superior to the criterion for seroprotection for each antigenic component for an acceptable percentage of human subjects. Antigens with an associated antibody titre above which a host is considered to be seroconverted against the antigen are well known, and such titres are published by organisations such as WHO. Preferably more than 80% of a statistically significant sample of subjects is seroconverted, more preferably more than 90%, still more preferably more than 93% and most preferably 96-100%.

Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intravenously,

intramuscularly, or to the interstitial space of a tissue), or by rectal, oral, vaginal, topical, transdermal, intranasal, ocular, aural, pulmonary or other mucosal administration. Intramuscular administration to the thigh or the upper arm is preferred. Injection may be via a needle (*e.g.* a hypodermic needle), but needle-free injection may alternatively be used. A typical intramuscular dose is 0.5 ml.

The invention may be used to elicit systemic and/or mucosal immunity.

Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. A primary dose schedule may be followed by a booster dose schedule. Suitable timing between priming doses (*e.g.* between 4-16 weeks), and between priming and boosting, can be routinely determined.

The immunogenic composition of the invention will generally include a pharmaceutically acceptable carrier, which can be any substance that does not itself induce the production of antibodies harmful to the patient receiving the composition, and which can be administered without undue toxicity. Suitable carriers can be large, slowly-metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Pharmaceutically acceptable carriers can include liquids such as water, saline, glycerol and ethanol. Auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, can also be present in such vehicles. Liposomes are suitable carriers. A thorough discussion of pharmaceutical carriers is available in ref. 14.

Neisserial infections affect various areas of the body and so the compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition be prepared for oral administration *e.g.* as a tablet or capsule, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as drops.

The composition is preferably sterile. It is preferably pyrogen-free. It is preferably buffered *e.g.* at between pH 6 and pH 8, generally around pH 7. Where a composition comprises an aluminium hydroxide salt, it is preferred to use a histidine buffer [15]. Compositions of the invention may be isotonic with respect to humans.

Immunogenic compositions comprise an immunologically effective amount of immunogen, as well as any other of other specified components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated

(*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. Dosage treatment may be a single dose schedule or a multiple dose schedule (*e.g.* including booster doses). The composition may be administered in conjunction with other immunoregulatory agents.

Adjuvants which may be used in compositions of the invention include, but are not limited to:

A. Mineral-containing compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphosphates, orthophosphates), sulphates, *etc.* [*e.g.* see chapters 8 & 9 of ref. 16], or mixtures of different mineral compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt [17].

Aluminium phosphates are particularly preferred, particularly in compositions which include a *H.influenzae* saccharide antigen, and a typical adjuvant is amorphous aluminium hydroxyphosphate with PO_4/Al molar ratio between 0.84 and 0.92, included at 0.6mg Al^{3+}/ml. Adsorption with a low dose of aluminium phosphate may be used *e.g.* between 50 and 100μg Al^{3+} per conjugate per dose. Where there is more than one conjugate in a composition, not all conjugates need to be adsorbed.

B. Oil Emulsions

Oil emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 [Chapter 10 of ref. 16; see also ref. 18] (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used.

C. Saponin formulations [chapter 22 of ref. 16]

Saponin formulations may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsaprilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs. QS21 is marketed as Stimulon™.

Saponin compositions have been purified using HPLC and RP-HPLC. Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in ref. 19. Saponin formulations may also comprise a sterol, such as cholesterol [20].

Combinations of saponins and cholesterols can be used to form unique particles called immunostimulating complexs (ISCOMs) [chapter 23 of ref. 16]. ISCOMs typically also include a

phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of QuilA, QHA & QHC. ISCOMs are further described in refs. 20-22. Optionally, the ISCOMS may be devoid of additional detergent [23].

A review of the development of saponin based adjuvants can be found in refs. 24 & 25.

D. Virosomes and virus-like particles

Virosomes and virus-like particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qß-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in refs. 26-31. Virosomes are discussed further in, for example, ref. 32

E. Bacterial or microbial derivatives

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), Lipid A derivatives, immunostimulatory oligonucleotides and ADP-ribosylating toxins and detoxified derivatives thereof.

Non-toxic derivatives of LPS include monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 de-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in ref. 33. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22μm membrane [33]. Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529 [34,35].

Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in refs. 36 & 37.

Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a dinucleotide sequence containing an unmethylated cytosine linked by a phosphate bond to a guanosine). Double-stranded RNAs and oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. References 38, 39 and 40 disclose possible analog substitutions *e.g.* replacement of guanosine with 2'-deoxy-7-deazaguanosine. The adjuvant effect of CpG oligonucleotides is further discussed in refs. 41-46.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT [47]. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it

may be more specific for inducing a B cell response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in refs. 48-50. Preferably, the CpG is a CpG-A ODN.

Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, refs. 47 & 51-53.

Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E.coli* (*E.coli* heat labile enterotoxin "LT"), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in ref. 54 and as parenteral adjuvants in ref. 55. The toxin or toxoid is preferably in the form of a holotoxin, comprising both A and B subunits. Preferably, the A subunit contains a detoxifying mutation; preferably the B subunit is not mutated. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LT-G192. The use of ADP-ribosylating toxins and detoxified derivaties thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in refs. 56-63. Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in ref. 64, specifically incorporated herein by reference in its entirety.

F. Human immunomodulators

Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 [65], *etc.*) [66], interferons (*e.g.* interferon-?), macrophage colony stimulating factor, and tumor necrosis factor.

G. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres [67] or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention [68].

H. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(a-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

I. Liposomes (Chapters 13 & 14 of ref. 16)

Examples of liposome formulations suitable for use as adjuvants are described in refs. 69-71.

J. Polyoxyethylene ether and polyoxyethylene ester formulations

Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters [72]. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol [73] as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol [74]. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-steoryl ether, polyoxytheylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

K. Polyphosphazene (PCPP)

PCPP formulations are described, for example, in refs. 75 and 76.

L. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

M. Imidazoquinolone Compounds.

Examples of imidazoquinolone compounds suitable for use adjuvants in the invention include Imiquamod and its homologues (*e.g.* "Resiquimod 3M"), described further in refs. 77 and 78.

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention: (1) a saponin and an oil-in-water emulsion [79]; (2) a saponin (*e.g.* QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) [80]; (3) a saponin (*e.g.* QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) + a cholesterol; (4) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) [81]; (5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions [82]; (6) SAF, containing 10% squalane, 0.4% Tween 80™, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion. (7) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); and (8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dMPL).

Other substances that act as immunostimulating agents are disclosed in chapter 7 of ref. 16.

Aluminium salts (aluminium phosphates and particularly hydroxyphosphates, and/or hydroxides and particularly oxyhydroxide) and MF59 are preferred adjuvants for parenteral immunisation. Toxin mutants are preferred mucosal adjuvants. QS21 is another useful adjuvant for NMB1870, which may be used alone or in combination with one or more other adjuvants *e.g.* with an aluminium salt.

Muramyl peptides include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), *etc.*

Further antigenic components

Compositions of the invention include chimeric NMB1870 polypeptides. It is particularly preferred that the composition should not include complex or undefined mixtures of antigens *e.g.* it is preferred not to include outer membrane vesicles in the composition. Polypeptides of the invention are preferably expressed recombinantly in a heterologous host and then purified.

The composition of the invention includes a chimeric NMB1870 polypeptide. It may also include one or more further neisserial antigen(s), as a vaccine which targets more than one antigen per bacterium decreases the possibility of selecting escape mutants. Neisserial antigens for inclusion in the compositions include proteins comprising:

(a) the 446 even SEQ IDs (*i.e.* 2, 4, 6, ... , 890, 892) disclosed in reference 83.

(b) the 45 even SEQ IDs (i.e. 2, 4, 6, ... , 88, 90) disclosed in reference 84;

(c) the 1674 even SEQ IDs 2-3020, even SEQ IDs 3040-3114, and all SEQ IDs 3115-3241, disclosed in reference 2;

(d) the 2160 amino acid sequences NMB0001 to NMB2160 from reference 4;

(e) a meningococcal PorA protein, of any subtype, preferably recombinantly expressed;

(f) a variant, homolog, ortholog, paralog, mutant *etc.* of (a) to (e); or

(g) an outer membrane vesicle preparation from *N.meningitidis* [*e.g.* see ref. 177].

In addition to Neisserial protein antigens, the composition may include antigens for immunising against other diseases or infections. For example, the composition may include one or more of the following further antigens:

- a saccharide antigen from *N.meningitidis* serogroup A, C, W135 and/or Y, such as the oligosaccharide disclosed in ref. 85 from serogroup C [see also ref. 86] or the oligosaccharides of ref. 87.
- a saccharide antigen from *Streptococcus pneumoniae* [*e.g.* 88, 89, 90].
- an antigen from hepatitis A virus, such as inactivated virus [*e.g.* 91, 92].
- an antigen from hepatitis B virus, such as the surface and/or core antigens [*e.g.* 92, 93].
- a diphtheria antigen, such as a diphtheria toxoid [*e.g.* chapter 3 of ref. 94] *e.g.* the CRM_{197} mutant [*e.g.* 95].
- a tetanus antigen, such as a tetanus toxoid [*e.g.* chapter 4 of ref. 94].
- an antigen from *Bordetella pertussis*, such as pertussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B.pertussis*, optionally also in combination with pertactin and/or agglutinogens 2 and 3 [*e.g.* refs. 96 & 97].
- a saccharide antigen from *Haemophilus influenzae* B [*e.g.* 86].
- polio antigen(s) [*e.g.* 98, 99] such as IPV.
- measles, mumps and/or rubella antigens [*e.g.* chapters 9, 10 & 11 of ref. 94].

- influenza antigen(s) [*e.g.* chapter 19 of ref. 94], such as the haemagglutinin and/or neuraminidase surface proteins.
- an antigen from *Moraxella catarrhalis* [*e.g.* 100].
- an protein antigen from *Streptococcus agalactiae* (group B streptococcus) [*e.g.* 101, 102].
- a saccharide antigen from *Streptococcus agalactiae* (group B streptococcus).
- an antigen from *Streptococcus pyogenes* (group A streptococcus) [*e.g.* 102, 103, 104].
- an antigen from *Staphylococcus aureus* [*e.g.* 105].

The composition may comprise one or more of these further antigens.

Toxic protein antigens may be detoxified where necessary (*e.g.* detoxification of pertussis toxin by chemical and/or genetic means [97]).

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens. DTP combinations are thus preferred.

Saccharide antigens are preferably in the form of conjugates. Carrier proteins for the conjugates include the *N.meningitidis* outer membrane protein [106], synthetic peptides [107,108], heat shock proteins [109,110], pertussis proteins [111,112], protein D from *H.influenzae* [113], cytokines [114], lymphokines [114], streptococcal proteins, hormones [114], growth factors [114], toxin A or B from *C.difficile* [115], iron-uptake proteins [116], *etc.* A preferred carrier protein is the CRM197 diphtheria toxoid [117].

Antigens in the composition will typically be present at a concentration of at least 1μg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

Immunogenic compositions of the invention may be used therapeutically (*i.e.* to treat an existing infection) or prophylactically (*i.e.* to prevent future infection).

As an alternative to using proteins antigens in the immunogenic compositions of the invention, nucleic acid (preferably DNA *e.g.* in the form of a plasmid) encoding the antigen may be used.

Particularly preferred compositions of the invention include one, two or three of: (a) saccharide antigens from meningococcus serogroups Y, W135, C and (optionally) A; (b) a saccharide antigen from *Haemophilus influenzae* type B; and/or (c) an antigen from *Streptococcus pneumoniae.*

Meningococcus serogroups Y, W135, C and (optionally) A

Polysaccharide vaccines against serogroups A, C, W135 & Y have been known for many years. These vaccines (MENCEVAX ACWY™ and MENOMUNE™) are based on the organisms' capsular polysaccharides and, although they are effective in adolescents and adults, they give a poor immune response and short duration of protection, and they cannot be used in infants.

In contrast to the unconjugated polysaccharide antigens in these vaccines, the recently-approved serogroup C vaccines (Menjugate™ [118,85], Meningitec™ and NeisVac-C™) include conjugated

saccharides. Menjugate™ and Meningitec™ have oligosaccharide antigens conjugated to a CRM_{197} carrier, whereas NeisVac-C™ uses the complete polysaccharide (de-O-acetylated) conjugated to a tetanus toxoid carrier. The Menactra™ vaccine contains conjugated capsular saccharide antigens from each of serogroups Y, W135, C and A.

Compositions of the present invention preferably include capsular saccharide antigens from one or more of meningococcus serogroups Y, W135, C and (optionally) A, wherein the antigens are conjugated to carrier protein(s) and/or are oligosaccharides. For example, the composition may include a capsular saccharide antigen from: serogroup C; serogroups A and C; serogroups A, C and W135; serogroups A, C and Y; serogroups C, W135 and Y; or from all four of serogroups A, C, W135 and Y.

A typical quantity of each meningococcal saccharide antigen per dose is between 1μg and 20μg *e.g.* about 1μg, about 2.5μg, about 4μg, about 5μg, or about 10μg (expressed as saccharide).

Where a mixture comprises capsular saccharides from both serogroups A and C, the ratio (w/w) of MenA saccharide:MenC saccharide may be greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Where a mixture comprises capsular saccharides from serogroup Y and one or both of serogroups C and W135, the ratio (w/w) of MenY saccharide:MenW135 saccharide may be greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher) and/or that the ratio (w/w) of MenY saccharide:MenC saccharide may be less than 1 (*e.g.* 1:2, 1:3, 1:4, 1:5, or lower). Preferred ratios (w/w) for saccharides from serogroups A:C:W135:Y are: 1:1:1:1; 1:1:1:2; 2:1:1:1; 4:2:1:1; 8:4:2:1; 4:2:1:2; 8:4:1:2; 4:2:2:1; 2:2:1:1; 4:4:2:1; 2:2:1:2; 4:4:1:2; and 2:2:2:1. Preferred ratios (w/w) for saccharides from serogroups C:W135:Y are: 1:1:1; 1:1:2; 1:1:1; 2:1:1; 4:2:1; 2:1:2; 4:1:2; 2:2:1; and 2:1:1. Using a substantially equal mass of each saccharide is preferred.

Capsular saccharides will generally be used in the form of oligosaccharides. These are conveniently formed by fragmentation of purified capsular polysaccharide (*e.g.* by hydrolysis), which will usually be followed by purification of the fragments of the desired size.

Fragmentation of polysaccharides is preferably performed to give a final average degree of polymerisation (DP) in the oligosaccharide of less than 30 (*e.g.* between 10 and 20, preferably around 10 for serogroup A; between 15 and 25 for serogroups W135 and Y, preferably around 15-20; between 12 and 22 for serogroup C; *etc.*). DP can conveniently be measured by ion exchange chromatography or by colorimetric assays [119].

If hydrolysis is performed, the hydrolysate will generally be sized in order to remove short-length oligosaccharides [86]. This can be achieved in various ways, such as ultrafiltration followed by ion-exchange chromatography. Oligosaccharides with a degree of polymerisation of less than or equal to about 6 are preferably removed for serogroup A, and those less than around 4 are preferably removed for serogroups W135 and Y.

Preferred MenC saccharide antigens are disclosed in reference 118, as used in Menjugate™.

The saccharide antigen may be chemically modified. This is particularly useful for reducing hydrolysis for serogroup A [120; see below]. De-O-acetylation of meningococcal saccharides can be performed. For oligosaccharides, modification may take place before or after depolymerisation.

Where a composition of the invention includes a MenA saccharide antigen, the antigen is preferably a modified saccharide in which one or more of the hydroxyl groups on the native saccharide has/have been replaced by a blocking group [120]. This modification improves resistance to hydrolysis.

The number of monosaccharide units having blocking groups can vary. For example, all or substantially all the monosaccharide units may have blocking groups. Alternatively, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the monosaccharide units may have blocking groups. At least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 monosaccharide units may have blocking groups.

Likewise, the number of blocking groups on a monosaccharide unit may vary. For example, the number of blocking groups on a monosaccharide unit may be 1 or 2. The blocking group will generally be at the 4 position and/or 3-position of the monosaccharide units.

The terminal monosaccharide unit may or may not have a blocking group instead of its native hydroxyl. It is preferred to retain a free anomeric hydroxyl group on a terminal monosaccharide unit in order to provide a handle for further reactions (*e.g.* conjugation). Anomeric hydroxyl groups can be converted to amino groups ($-NH_2$ or –NH-E, where E is a nitrogen protecting group) by reductive amination (using, for example, $NaBH_3CN/NH_4Cl$), and can then be regenerated after other hydroxyl groups have been converted to blocking groups.

Blocking groups to replace hydroxyl groups may be directly accessible via a derivatizing reaction of the hydroxyl group *i.e.* by replacing the hydrogen atom of the hydroxyl group with another group. Suitable derivatives of hydroxyl groups which act as blocking groups are, for example, carbamates, sulfonates, carbonates, esters, ethers (*e.g.* silyl ethers or alkyl ethers) and acetals. Some specific examples of such blocking groups are allyl, Aloc, benzyl, BOM, t-butyl, trityl, TBS, TBDPS, TES, TMS, TIPS, PMB, MEM, MOM, MTM, THP, *etc.* Other blocking groups that are not directly accessible and which completely replace the hydroxyl group include C_{1-12} alkyl, C_{3-12} alkyl, C_{5-12} aryl, C_{5-12} aryl-C_{1-6} alkyl, NR^1R^2 (R^1 and R^2 are defined in the following paragraph), H, F, Cl, Br, CO_2H, $CO_2(C_{1-6}$ alkyl), CN, CF_3, CCl_3, *etc.* Preferred blocking groups are electron-withdrawing groups.

Preferred blocking groups are of the formula: –O–X–Y or $-OR^3$ wherein: X is C(O), S(O) or SO_2; Y is C_{1-12} alkyl, C_{1-12} alkoxy, C_{3-12} cycloalkyl, C_{5-12} aryl or C_{5-12} aryl-C_{1-6} alkyl, each of which may optionally be substituted with 1, 2 or 3 groups independently selected from F, Cl, Br, CO_2H, $CO_2(C_{1-6}$ alkyl), CN, CF_3 or CCl_3; or Y is NR^1R^2; R^1 and R^2 are independently selected from H, C_{1-12} alkyl, C_{3-12} cycloalkyl, C_{5-12} aryl, C_{5-12} aryl-C_{1-6} alkyl; or R^1 and R^2 may be joined to form a C_{3-12} saturated heterocyclic group; R^3 is C_{1-12} alkyl or C_{3-12} cycloalkyl, each of which may optionally be substituted with 1, 2 or 3 groups independently selected from F, Cl, Br, $CO_2(C_{1-6}$ alkyl), CN, CF_3 or CCl_3; or R^3 is C_{5-12} aryl or C_{5-12} aryl-C_{1-6} alkyl, each of which may optionally be substituted with 1, 2, 3, 4 or 5

groups selected from F, Cl, Br, CO_2H, $CO_2(C_{1-6}$ alkyl), CN, CF_3 or CCl_3. When R^3 is C_{1-12} alkyl or C_{3-12} cycloalkyl, it is typically substituted with 1, 2 or 3 groups as defined above. When R^1 and R^2 are joined to form a C_{3-12} saturated heterocyclic group, it is meant that R^1 and R^2 together with the nitrogen atom form a saturated heterocyclic group containing any number of carbon atoms between 3 and 12 (*e.g.* C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}). The heterocyclic group may contain 1 or 2 heteroatoms (such as N, O or S) other than the nitrogen atom. Examples of C_{3-12} saturated heterocyclic groups are pyrrolidinyl, piperidinyl, morpholinyl, piperazinyl, imidazolidinyl, azetidinyl and aziridinyl.

Blocking groups -O-X-Y and $-OR^3$ can be prepared from -OH groups by standard derivatizing procedures, such as reaction of the hydroxyl group with an acyl halide, alkyl halide, sulfonyl halide, *etc.* Hence, the oxygen atom in -O-X-Y is preferably the oxygen atom of the hydroxyl group, while the -X-Y group in -O-X-Y preferably replaces the hydrogen atom of the hydroxyl group.

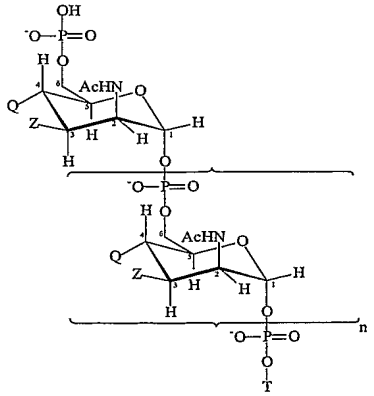
Alternatively, the blocking groups may be accessible via a substitution reaction, such as a Mitsonobu-type substitution. These and other methods of preparing blocking groups from hydroxyl groups are well known.

More preferably, the blocking group is $-OC(O)CF_3$ [121], or a carbamate group $-OC(O)NR^1R^2$, where R^1 and R^2 are independently selected from C_{1-6} alkyl. More preferably, R^1 and R^2 are both methyl *i.e.* the blocking group is $-OC(O)NMe_2$. Carbamate blocking groups have a stabilizing effect on the glycosidic bond and may be prepared under mild conditions.

Preferred modified MenA saccharides contain *n* monosaccharide units, where at least *h%* of the monosaccharide units do not have –OH groups at both of positions 3 and 4. The value of *h* is 24 or more (*e.g.* 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 98, 99 or 100) and is preferably 50 or more. The absent –OH groups are preferably blocking groups as defined above.

Other preferred modified MenA saccharides comprise monosaccharide units, wherein at least *s* of the monosaccharide units do not have–OH at the 3 position and do not have –OH at the 4 position. The value of *s* is at least 1 (*e.g.* 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 60, 70, 80, 90). The absent –OH groups are preferably blocking groups as defined above.

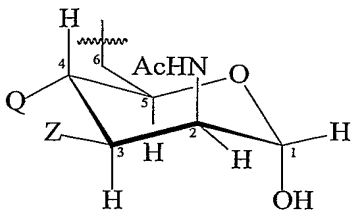
Suitable modified MenA saccharides for use with the invention have the formula:



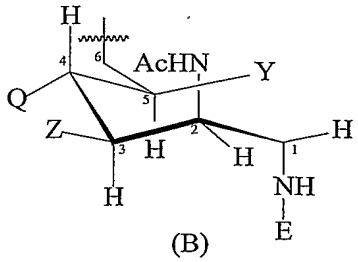
, wherein

n is an integer from 1 to 100 (preferably an integer from 15 to 25);

T is of the formula (A) or (B):



(A)



(B)

each Z group is independently selected from OH or a blocking group as defined above; and

each Q group is independently selected from OH or a blocking group as defined above;

Y is selected from OH or a blocking group as defined above;

E is H or a nitrogen protecting group;

and wherein more than about 7% (*e.g.* 8%, 9%, 10% or more) of the Q groups are blocking groups.

Each of the *n+2* Z groups may be the same or different from each other. Likewise, each of the *n+2* Q groups may be the same or different from each other. All the Z groups may be OH. Alternatively, at least 10%, 20, 30%, 40%, 50% or 60% of the Z groups may be OAc. Preferably, about 70% of the Z groups are OAc, with the remainder of the Z groups being OH or blocking groups as defined above. At least about 7% of Q groups are blocking groups. Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or even 100% of the Q groups are blocking groups.

Meningococcal capsular polysaccharides are typically prepared by a process comprising the steps of polysaccharide precipitation (*e.g.* using a cationic detergent), ethanol fractionation, cold phenol extraction (to remove protein) and ultracentrifugation (to remove LPS) [*e.g.* ref. 122]. A more preferred process [87], however, involves polysaccharide precipitation followed by solubilisation of the precipitated polysaccharide using a lower alcohol. Precipitation can be achieved using a cationic detergent such as tetrabutylammonium and cetyltrimethylammonium salts (*e.g.* the bromide salts), or hexadimethrine bromide and myristyltrimethylammonium salts. Cetyltrimethylammonium bromide ('CTAB') is particularly preferred [123]. Solubilisation of the precipitated material can be achieved using a lower alcohol such as methanol, propan-1-ol, propan-2-ol, butan-1-ol, butan-2-ol, 2-methyl-propan-1-ol, 2-methyl-propan-2-ol, diols, *etc.*, but ethanol is particularly suitable for solubilising CTAB-polysaccharide complexes. Ethanol is preferably added to the precipitated polysaccharide to give a final concentration (based on total content of ethanol and water) of between 50% and 95%.

After re-solubilisation, the polysaccharide may be further treated to remove contaminants. This is particularly important in situations where even minor contamination is not acceptable (*e.g.* for human vaccine production). This will typically involve one or more steps of filtration *e.g.* depth filtration, filtration through activated carbon may be used, size filtration and/or ultrafiltration. Once filtered to remove contaminants, the polysaccharide may be precipitated for further treatment and/or processing. This can be conveniently achieved by exchanging cations (*e.g.* by the addition of calcium or sodium salts).

As an alternative to purification, capsular saccharides may be obtained by total or partial synthesis *e.g.* Hib synthesis is disclosed in ref. 124, and MenA synthesis in ref. 125.

Compositions of the invention comprise capsular saccharides from at least two serogroups of *N.meningitidis*. The saccharides are preferably prepared separately (including any fragmentation, conjugation, modification, *etc.*) and then admixed to give a composition of the invention.

Where the composition comprises capsular saccharide from serogroup A, however, it is preferred that the serogroup A saccharide is not combined with the other saccharide(s) until shortly before use, in order to minimise the potential for hydrolysis. This can conveniently be achieved by having the serogroup A component (typically together with appropriate excipients) in lyophilised form and the other serogroup component(s) in liquid form (also with appropriate excipients), with the liquid components being used to reconstitute the lyophilised MenA component when ready for use. Where an aluminium salt adjuvant is used, it is preferred to include the adjuvant in the vial containing the with the liquid vaccine, and to lyophilise the MenA component without adjuvant.

A composition of the invention may thus be prepared from a kit comprising: (a) capsular saccharide from *N.meningitidis* serogroup A, in lyophilised form; and (b) the further antigens from the composition, in liquid form. The invention also provides a method for preparing a composition of the invention, comprising mixing a lyophilised capsular saccharide from *N.meningitidis* serogroup A with the further antigens, wherein said further antigens are in liquid form.

The invention also provides a kit comprising: (a) a first container containing capsular saccharides from two or more of *N.meningitidis* serogroups C, W135 and Y, all in lyophilised form; and (b) a second container containing in liquid form (i) a composition which, after administration to a subject, is able to induce an antibody response in that subject, wherein the antibody response is bactericidal against two or more (*e.g.* 2 or 3) of hypervirulent lineages A4, ET-5 and lineage 3 of *N.meningitidis* serogroup B, (ii) capsular saccharides from none or one of *N.meningitidis* serogroups C, W135 and Y, and optionally (iii) further antigens (see below) that do not include meningococcal capsular saccharides, wherein, reconstitution of the contents of container (a) by the contents of container (b) provides a composition of the invention.

Within each dose, the amount of an individual saccharide antigen will generally be between 1-50 μg (measured as mass of saccharide), with about 2.5μg, 5μg or 10 μg of each being preferred. With A:C:W135:Y weight ratios of 1:1:1:1; 1:1:1:2; 2:1:1:1; 4:2:1:1; 8:4:2:1; 4:2:1:2; 8:4:1:2; 4:2:2:1; 2:2:1:1; 4:4:2:1; 2:2:1:2; 4:4:1:2; and 2:2:2:1, therefore, the amount represented by the number 1 is preferably about 2.5μg, 5μg or 10 μg. For a 1:1:1:1 ratio A:C:W:Y composition and a 10μg per saccharide, therefore, 40 μg saccharide is administered per dose. Preferred compositions have about the following μg saccharide per dose:

A	10	0	0	0	10	5	2.5
C	10	10	5	2.5	5	5	2.5
W135	10	10	5	2.5	5	5	2.5
Y	10	10	5	2.5	5	5	2.5

Preferred compositions of the invention comprise less than 50 μg meningococcal saccharide per dose. Other preferred compositions comprise ≤40 μg meningococcal saccharide per dose. Other preferred compositions comprise ≤30 μg meningococcal saccharide per dose. Other preferred compositions comprise ≤25 μg meningococcal saccharide per dose. Other preferred compositions comprise ≤20 μg meningococcal saccharide per dose. Other preferred compositions comprise ≤10 μg meningococcal saccharide per dose but, ideally, compositions of the invention comprise at least 10 μg meningococcal saccharide per dose.

The Menjugate™ and NeisVac™ MenC conjugates use a hydroxide adjuvant, whereas Meningitec™ uses a phosphate. It is possible in compositions of the invention to adsorb some antigens to an aluminium hydroxide but to have other antigens in association with an aluminium phosphate. For tetravalent serogroup combinations, for example, the following permutations are available:

Serogroup	**Aluminium salt** (H = a hydroxide; P = a phosphate)															
A	P	H	P	H	H	H	P	P	P	H	H	H	P	P	P	H
C	P	H	H	P	H	H	P	H	H	P	P	H	P	H	P	P
W135	P	H	H	H	P	H	H	P	H	H	P	P	P	P	H	P
Y	P	H	H	H	H	P	H	H	P	P	H	P	H	P	P	P

For trivalent *N.meningitidis* serogroup combinations, the following permutations are available:

Serogroup	**Aluminium salt** (H = a hydroxide; P = a phosphate)							
C	P	H	H	H	P	P	P	H
W135	P	H	H	P	H	P	H	P
Y	P	H	P	H	H	H	P	P

Haemophilus influenzae type B

Where the composition includes a *H.influenzae* type B antigen, it will typically be a Hib capsular saccharide antigen. Saccharide antigens from *H.influenzae* b are well known.

Advantageously, the Hib saccharide is covalently conjugated to a carrier protein, in order to enhance its immunogenicity, especially in children. The preparation of polysaccharide conjugates in general, and of the Hib capsular polysaccharide in particular, is well documented [*e.g.* references 126 to 134 *etc.*]. The invention may use any suitable Hib conjugate. Suitable carrier proteins are described below, and preferred carriers for Hib saccharides are CRM_{197} ('HbOC'), tetanus toxoid ('PRP-T') and the outer membrane complex of *N.meningitidis* ('PRP-OMP').

The saccharide moiety of the conjugate may be a polysaccharide (*e.g.* full-length polyribosylribitol phosphate (PRP)), but it is preferred to hydrolyse polysaccharides to form oligosaccharides (*e.g.* MW from ~1 to ~5 kDa).

A preferred conjugate comprises a Hib oligosaccharide covalently linked to CRM_{197} via an adipic acid linker [135, 136]. Tetanus toxoid is also a preferred carrier.

Compositions of the invention may comprise more than one Hib antigen.

Where a composition includes a Hib saccharide antigen, it is preferred that it does not also include an aluminium hydroxide adjuvant. If the composition includes an aluminium phosphate adjuvant then the Hib antigen may be adsorbed to the adjuvant [137] or it may be non-adsorbed [138].

Hib antigens may be lyophilised *e.g.* together with meningococcal antigens.

Streptococcus pneumoniae

Where the composition includes a *S.pneumoniae* antigen, it will typically be a capsular saccharide antigen which is preferably conjugated to a carrier protein [*e.g.* refs. 88-90]. It is preferred to include saccharides from more than one serotype of *S.pneumoniae*. For example, mixtures of polysaccharides from 23 different serotype are widely used, as are conjugate vaccines with polysaccharides from between 5 and 11 different serotypes [139]. For example, PrevNar™ [140] contains antigens from seven serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) with each saccharide individually conjugated to CRM_{197} by reductive amination, with 2μg of each saccharide per 0.5ml dose (4μg of serotype 6B), and with conjugates adsorbed on an aluminium phosphate adjuvant. Compositions of the invention preferably include at least serotypes 6B, 14, 19F and 23F. Conjugates may be adsorbed onto an aluminium phosphate.

As an alternative to using saccharide antigens from pneumococcus, the composition may include one or more polypeptide antigens. Genome sequences for several strains of pneumococcus are available [141,142] and can be subjected to reverse vaccinology [143-146] to identify suitable polypeptide antigens [147,148]. For example, the composition may include one or more of the following antigens: PhtA, PhtD, PhtB, PhtE, SpsA, LytB, LytC, LytA, Sp125, Sp101, Sp128 and Sp130, as defined in reference 149.

In some embodiments, the composition may include both saccharide and polypeptide antigens from pneumococcus. These may be used in simple admixture, or the pneumococcal saccharide antigen may be conjugated to a pneumococcal protein. Suitable carrier proteins for such embodiments include the antigens listed in the previous paragraph [149].

Pneumococcal antigens may be lyophilised *e.g.* together with meningococcal and/or Hib antigens.

Covalent conjugation

Capsular saccharides in compositions of the invention will usually be conjugated to carrier protein(s). In general, conjugation enhances the immunogenicity of saccharides as it converts them from T-independent antigens to T-dependent antigens, thus allowing priming for immunological memory. Conjugation is particularly useful for paediatric vaccines and is a well known technique [*e.g.* reviewed in refs. 150 and 126-134].

Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria toxoid or tetanus toxoid. The CRM_{197} mutant diphtheria toxin [117,151,152] is particularly preferred. Other suitable carrier proteins include the *N.meningitidis* outer membrane protein [106], synthetic peptides [107,108], heat shock proteins [109,110], pertussis proteins [111,112], cytokines [114], lymphokines [114], hormones [114], growth factors [114], artificial proteins comprising multiple human $CD4^+$ T cell epitopes from various pathogen-derived antigens [153], protein D from *H.influenzae* [113,154], pneumococcal surface protein PspA [155], iron-uptake proteins [116], toxin A or B from *C.difficile* [115], *etc.* Preferred carriers are diphtheria toxoid, tetanus toxoid, *H.influenzae* protein D, and CRM_{197}.

Within a composition of the invention, it is possible to use more than one carrier protein *e.g.* to reduce the risk of carrier suppression. Thus different carrier proteins can be used for different serogroups *e.g.* serogroup A saccharides might be conjugated to CRM_{197} while serogroup C saccharides might be conjugated to tetanus toxoid. It is also possible to use more than one carrier protein for a particular saccharide antigen *e.g.* serogroup A saccharides might be in two groups, with some conjugated to CRM_{197} and others conjugated to tetanus toxoid. In general, however, it is preferred to use the same carrier protein for all saccharides.

A single carrier protein might carry more than one saccharide antigen [156]. For example, a single carrier protein might have conjugated to it saccharides from serogroups A and C. To achieve this goal, saccharides can be mixed prior to the conjugation reaction. In general, however, it is preferred to have separate conjugates for each serogroup.

Conjugates with a saccharide:protein ratio (w/w) of between 1:5 (*i.e.* excess protein) and 5:1 (*i.e.* excess saccharide) are preferred. Ratios between 1:2 and 5:1 are preferred, as are ratios between 1:1.25 and 1:2.5 are more preferred. Excess carrier protein is preferred for MenA and MenC.

Conjugates may be used in conjunction with free carrier protein [157]. When a given carrier protein is present in both free and conjugated form in a composition of the invention, the unconjugated form is preferably no more than 5% of the total amount of the carrier protein in the composition as a whole, and more preferably present at less than 2% by weight.

Any suitable conjugation reaction can be used, with any suitable linker where necessary.

The saccharide will typically be activated or functionalised prior to conjugation. Activation may involve, for example, cyanylating reagents such as CDAP (*e.g.* 1-cyano-4-dimethylamino pyridinium tetrafluoroborate [158,159,*etc.*]). Other suitable techniques use carbodiimides, hydrazides, active esters, norborane, p-nitrobenzoic acid, N-hydroxysuccinimide, S-NHS, EDC, TSTU; see also the introduction to reference 132).

Linkages via a linker group may be made using any known procedure, for example, the procedures described in references 160 and 161. One type of linkage involves reductive amination of the polysaccharide, coupling the resulting amino group with one end of an adipic acid linker group, and then coupling a protein to the other end of the adipic acid linker group [130,162,163]. Other linkers

include B-propionamido [164], nitrophenyl-ethylamine [165], haloacyl halides [166], glycosidic linkages [167], 6-aminocaproic acid [168], ADH [169], C_4 to C_{12} moieties [170] *etc.* As an alternative to using a linker, direct linkage can be used. Direct linkages to the protein may comprise oxidation of the polysaccharide followed by reductive amination with the protein, as described in, for example, references 171 and 172.

A process involving the introduction of amino groups into the saccharide (*e.g.* by replacing terminal =O groups with $-NH_2$) followed by derivatisation with an adipic diester (*e.g.* adipic acid N-hydroxysuccinimido diester) and reaction with carrier protein is preferred. Another preferred reaction uses CDAP activation with a protein D carrier *e.g.* for MenA or MenC.

After conjugation, free and conjugated saccharides can be separated. There are many suitable methods, including hydrophobic chromatography, tangential ultrafiltration, diafiltration *etc.* [see also refs. 173 & 174, *etc.*].

Where the composition of the invention includes a conjugated oligosaccharide, it is preferred that oligosaccharide preparation precedes conjugation.

Outer membrane vesicles

It is preferred that compositions of the invention should not include complex or undefined mixtures of antigens, which are typical characteristics of OMVs. However, one way in which the invention can be applied to OMVs is where OMVs are to be administered in a multiple dose schedule.

Where more than one OMV dose is to be administered, each dose may be supplemented (either by adding the purified protein or by expression of the protein within the bacteria from which the OMVs are derived) by one of the first protein, second protein or third protein as defined above. Preferably different doses are supplemented with different NMB1870 families. In a three dose OMV schedule, for example, each dose could contain a different one of the first protein, second protein and third protein such that, after receiving three doses of OMVs, all three families have been received. In a two dose OMV schedule, one family could be used per OMV dose (thus omitting one family), or one or both OMV doses could be supplemented with more than one family in order to give coverage with all three families. In preferred embodiments, there are three OMV doses, and each of the three OMV doses contains three different genetically-engineered vesicle populations each displaying three subtypes, thereby giving nine different subtypes in all.

This approach may be used in general to improve preparations of *N.meningitidis* serogroup B microvesicles [175], 'native OMVs' [176], blebs or outer membrane vesicles [*e.g.* refs. 177 to 182, *etc.*]. These may be prepared from bacteria which have been genetically manipulated [183-186] *e.g.* to increase immunogenicity (*e.g.* hyper-express immunogens), to reduce toxicity, to inhibit capsular polysaccharide synthesis, to down-regulate PorA expression, *etc.* They may be prepared from hyperblebbing strains [187-190]. Vesicles from a non-pathogenic *Neisseria* may be included [191]. OMVs may be prepared without the use of detergents [192,193]. They may express non-Neisserial proteins on their surface [194]. They may be LPS-depleted. They may be mixed with recombinant

antigens [177,195]. Vesicles from bacteria with different class I outer membrane protein subtypes may be used *e.g.* six different subtypes [196,197] using two different genetically-engineered vesicle populations each displaying three subtypes, or nine different subtypes using three different genetically-engineered vesicle populations each displaying three subtypes, *etc.* Useful subtypes include: P1.7,16; P1.5-1,2-2; P1.19,15-1; P1.5-2,10; P1.12-1,13; P1.7-2,4; P1.22,14; P1.7-1,1; P1.18-1,3,6.

It is also possible, of course, to supplement vesicle preparations with two or three different families.

Monoclonal antibodies

The invention provides a monoclonal antibody that recognises an epitope in a meningococcal NMB1870 protein, wherein said epitope requires the presence of both domains B and C in said NMB1870. Thus the monoclonal antibody does not bind to a separate domain B or to a separate domain C, but it does bind to a combination of domains B and C (and also to full NMB1870). Thus the epitope may be a discontinuous epitope formed from amino acid residues in both domain B and domain C.

The term 'monoclonal antibody' includes any of the various artificial antibodies and antibody-derived proteins which are available *e.g.* human antibodies, chimeric antibodies, humanized antibodies, single-domain antibodies, single-chain Fv (scFV) antibodies, monoclonal oligobodies, dimeric or trimeric antibody fragments or constructs, minibodies, or functional fragments thereof which bind to the antigen in question. The antibody is preferably in substantially isolated form.

In a natural antibody molecule, there are two heavy chains and two light chains. Each heavy chain and each light chain has at its N-terminal end a variable domain. Each variable domain is composed of four framework regions (FRs) alternating with three complementarity determining regions (CDRs). The residues in the variable domains are conventionally numbered according to a system devised by Kabat *et al.* [198]. The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues and the linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering. This may correspond to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure.

To avoid a non-specific anti-mouse immune response in humans, non-human antibodies are preferably humanized or chimeric. [*e.g.* refs. 199 & 200]. As an alternative, fully-human antibodies may be used. In chimeric antibodies, non-human constant regions are substituted by human constant regions but variable regions remain non-human. Humanized antibodies may be achieved by a variety of methods including, for example: (1) grafting complementarity determining regions (CDRs) from the non-human variable region onto a human framework ("CDR-grafting"), with the optional additional transfer of one or more framework residues from the non-human antibody ("humanizing"); (2) transplanting entire non-human variable domains, but "cloaking" them with a human-like surface by replacement of surface residues ("veneering"). In the present invention, humanized antibodies

include those obtained by CDR-grafting, humanizing, and veneering of the variable regions. [*e.g.* refs. 201 to 207].

Humanized or fully-human antibodies can also be produced using transgenic animals that are engineered to contain human immunoglobulin loci. For example, ref. 208 discloses transgenic animals having a human Ig locus wherein the animals do not produce functional endogenous immunoglobulins due to the inactivation of endogenous heavy and light chain loci. Ref. 209 also discloses transgenic non-primate mammalian hosts capable of mounting an immune response to an immunogen, wherein the antibodies have primate constant and/or variable regions, and wherein the endogenous immunoglobulin-encoding loci are substituted or inactivated. Ref. 210 discloses the use of the Cre/Lox system to modify the immunoglobulin locus in a mammal, such as to replace all or a portion of the constant or variable region to form a modified antibody molecule. Ref. 211 discloses non-human mammalian hosts having inactivated endogenous Ig loci and functional human Ig loci. Ref. 212 discloses methods of making transgenic mice in which the mice lack endogenous heavy chains, and express an exogenous immunoglobulin locus comprising one or more xenogeneic constant regions.

Antibodies naturally have two separate chains, however, it is preferred to use a single chain antibody ("sFv") in which the light and heavy chain variable domains are joined by a linker to give a single polypeptide chain. Kits for preparing sFv's are available off-the-shelf, and anti-ligand sFvs are preferred second sequences for use with the invention. Single domain antibodies can also be obtained from camelids or sharks [213], or by camelisation [214].

A sFv polypeptide is a covalently linked V_H-V_L heterodimer which is expressed from a gene fusion including V_H-and V_L- encoding genes linked by a peptide-encoding linker [215]. A number of methods have been described to discern and develop chemical strucutres (linkers) for converting the naturally aggregated, but chemically separated, light and heavy polypeptide chains from an antibody V region into an sFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, *e.g.*, refs. 216-218. The sFv molecules may be produced using methods described in the art. Design criteria include determining the appropriate length to span the distance between the C-terminus of one chain and the N-terminus of the other, wherein the linker is generally formed from small hydrophilic amino acid residues that do not coil or form secondary structures. Such methods have been described in the art [*e.g.* refs. 216-218]. Suitable linkers generally comprise polypeptide chains of alternating sets of glycine and serine residues, and may include glutamic acid and lysine residues inserted to enhance solubility.

"Mini-antibodies" or "minibodies" will also find use with the present invention. Minibodies are sFv polypeptide chains which include oligomerization domains at their C-termini, separated from the sFv by a hinge region [219]. The oligomerization domain comprises self-associating α-helices, *e.g.*, leucine zippers, that can be further stabilized by additional disulfide bonds. The oligomerization domain is designed to be compatible with vectorial folding across a membrane, a process thought to

facilitate in vivo folding of the polypeptide into a functional binding protein. Generally, minibodies are produced using recombinant methods well known in the art. See, *e.g* [219], [220].

"Oligobodies" will also find use with the present invention. Oligobodies are synthetic antibodies. The specificity of these reagents has been demonstrated by Western blot analysis and immunohistochemistry. They have some desirable properties, namely that as their production is independent of the immune system, they can be prepared in a few days and there is no need for a purified target protein [221]. Oligobodies are produced using recombinant methods well known in the art [222].

Antibodies are produced using techniques well known to those of skill in the art [*e.g.* refs. 223-228]. Monoclonal antibodies are generally prepared using the method of Kohler & Milstein (1975) [229], or a modification thereof. Typically, a mouse or rat is immunized as described above. Rabbits may also be used. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of non-specifically adherent cells) by applying a cell suspension to a plate or well coated with the antigen. B-cells, expressing membrane-bound immunoglobulin specific for the antigen, will bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (*e.g.* 'HAT' medium). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected monoclonal antibody-secreting hybridomas are then cultured either *in vitro* (*e.g.* in tissue culture bottles or hollow fiber reactors), or *in vivo* (*e.g.* as ascites in mice).

The invention also provides a hybridoma expressing the antibody of the invention. This hybridoma can be used in various ways *e.g.* as a source of monoclonal antibodies or as a source of nucleic acid (DNA or mRNA) encoding the monoclonal antibody of the invention for the cloning of antibody genes for subsequent recombinant expression.

Antibodies may be produced by any suitable means (*e.g.* by recombinant expression). Expression from recombinant sources is more common for pharmaceutical purposes than expression from B cells or hybridomas *e.g.* for reasons of stability, reproducibility, culture ease, *etc.*

Antibody fragments which retain the ability to recognise NMB1870 are also included within the scope of the invention. A number of antibody fragments are known in the art which comprise antigen-binding sites capable of exhibiting immunological binding properties of an intact antibody molecule. For example, functional antibody fragments can be produced by cleaving a constant region, not responsible for antigen binding, from the antibody molecule, using *e.g.*, pepsin, to produce $F(ab')_2$ fragments. These fragments will contain two antigen binding sites, but lack a portion of the constant region from each of the heavy chains. Similarly, if desired, Fab fragments, comprising a single antigen binding site, can be produced, *e.g.*, by digestion of monoclonal antibodies with papain. Functional fragments, including only the variable regions of the heavy and

light chains, can also be produced, using standard techniques such as recombinant production or preferential proteolytic cleavage of immunoglobulin molecules. These fragments are known as Fv. See, *e.g.*, [230], [231] and [232].

Non-conventional means can also be used to generate and identify the antibodies of the invention. For example, a phage display library can be screened for antibodies of the invention [233-236].

Antibodies of the invention can be of any isotype (*e.g.* IgA, IgG, IgM *i.e.* an α, γ or μ heavy chain), but will preferably be IgG. Within the IgG isotype, antibodies may be IgG1, IgG2, IgG3 or IgG4 subclass. Antibodies of the invention may have a κ or a λ light chain.

Protein expression

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*e.g.* structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in Escherichia coli (E. coli) [Raibaud *et al.* (1984) *Annu. Rev. Genet. 18*:173]. Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) [Chang *et al.* (1977) *Nature 198*:1056], and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) [Goeddel *et al.* (1980) *Nuc. Acids Res. 8*:4057; Yelverton *et al.* (1981) *Nucl. Acids Res. 9*:731; US patent 4,738,921; EP-A-0036776 and EP-A-0121775]. The ß-lactamase (*bla*) promoter system [Weissmann (1981) "The cloning of interferon and other mistakes." In *Interferon 3* (ed. I. Gresser)], bacteriophage lambda PL [Shimatake *et al.* (1981) *Nature 292*:128] and T5 [US patent 4,689,406] promoter systems also provide useful promoter sequences. Another promoter of interest is an inducible arabinose promoter (pBAD).

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter [US patent 4,551,433]. For example, the *tac* promoter is a hybrid *trp-lac* promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor [Amann *et al.* (1983) *Gene 25*:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci. 80*:21]. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in

prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system [Studier *et al.* (1986) *J. Mol. Biol. 189*:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci. 82*:1074]. In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO-A-0 267 851).

In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon [Shine *et al.* (1975) *Nature 254*:34]. The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' and of *E. coli* 16S rRNA [Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological Regulation and Development: Gene Expression* (ed. R.F. Goldberger)]. To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site [Sambrook *et al.* (1989) "Expression of cloned genes in Escherichia coli." In *Molecular Cloning: A Laboratory Manual*].

A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* on *in vitro* incubation with a bacterial methionine N-terminal peptidase (EP-A-0219237).

Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*e.g.* plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various Bacillus strains integrate into the Bacillus chromosome (EP-A-0127328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline [Davies *et al.* (1978) *Annu. Rev. Microbiol. 32*:469]. Selectable markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable market that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: Bacillus subtilis [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA 79*:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541], Escherichia coli [Shimatake *et al.* (1981) *Nature 292*:128; Amann *et al.* (1985) *Gene 40*:183; Studier *et al.* (1986) *J. Mol. Biol. 189*:113; EP-A-0 036 776,EP-A-0 136 829 and EP-A-0 136 907], Streptococcus cremoris [Powell *et al.* (1988) *Appl. Environ. Microbiol. 54*:655]; Streptococcus lividans [Powell *et al.* (1988) *Appl. Environ. Microbiol. 54*:655], Streptomyces lividans [US patent 4,745,056].

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with $CaCl_2$ or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. See *e.g.* [Masson *et al.* (1989) *FEMS Microbiol. Lett. 60*:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA 79*:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541, Bacillus], [Miller *et al.* (1988) *Proc. Natl. Acad. Sci. 85*:856; Wang *et al.* (1990) *J. Bacteriol. 172*:949, Campylobacter], [Cohen *et al.* (1973) *Proc. Natl. Acad. Sci. 69*:2110; Dower *et al.* (1988) *Nucleic Acids Res. 16*:6127; Kushner (1978) "An improved method for transformation of Escherichia coli with ColE1-derived plasmids. In *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol. 53*:159; Taketo (1988) *Biochim. Biophys. Acta 949*:318; Escherichia], [Chassy *et al.* (1987) *FEMS Microbiol. Lett. 44*:173 Lactobacillus]; [Fiedler *et al.* (1988) *Anal. Biochem 170*:38, Pseudomonas]; [Augustin *et al.* (1990) *FEMS Microbiol. Lett. 66*:203, Staphylococcus], [Barany *et al.* (1980) *J. Bacteriol. 144*:698; Harlander (1987) "Transformation of Streptococcus lactis by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun. 32*:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol. 54*:655; Somkuti *et al.* (1987) *Proc. 4th Evr. Cong. Biotechnology 1*:412, Streptococcus].

General

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value x means, for example, $x \pm 10\%$.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

"Sequence identity" is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters *gap open penalty=12* and *gap extension penalty=1*.

After serogroup, meningococcal classification includes serotype, serosubtype and then immunotype, and the standard nomenclature lists serogroup, serotype, serosubtype, and immunotype, each separated by a colon *e.g.* B:4:P1.15:L3,7,9. Within serogroup B, some lineages cause disease often (hyperinvasive), some lineages cause more severe forms of disease than others (hypervirulent), and others rarely cause disease at all. Seven hypervirulent lineages are recognised, namely subgroups I, III and IV-1, ET-5 complex, ET-37 complex, A4 cluster and lineage 3. These have been defined by multilocus enzyme electrophoresis (MLEE), but multilocus sequence typing (MLST) has also been used to classify meningococci [ref. 10]. The four main hypervirulent clusters are ST32, ST44, ST8 and ST11 complexes.

The term "alkyl" refers to alkyl groups in both straight and branched forms, The alkyl group may be interrupted with 1, 2 or 3 heteroatoms selected from -O-, -NH- or -S-. The alkyl group may also be interrupted with 1, 2 or 3 double and/or triple bonds. However, the term "alkyl" usually refers to alkyl groups having no heteroatom interruptions or double or triple bond interruptions. Where reference is made to C_{1-12} alkyl, it is meant the alkyl group may contain any number of carbon atoms between 1 and 12 (*e.g.* C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}). Similarly, where reference is made to C_{1-6} alkyl, it is meant the alkyl group may contain any number of carbon atoms between 1 and 6 (*e.g.* C_1, C_2, C_3, C_4, C_5, C_6).

The term "cycloalkyl" includes cycloalkyl, polycycloalkyl, and cycloalkenyl groups, as well as combinations of these with alkyl groups, such as cycloalkylalkyl groups. The cycloalkyl group may be interrupted with 1, 2 or 3 heteroatoms selected from -O-, -NH- or -S-. However, the term "cycloalkyl" usually refers to cycloalkyl groups having no heteroatom interruptions Examples of cycloalkyl groups include cyclopentyl, cyclohexyl, cyclohexenyl, cyclohexylmethyl and adamantyl groups. Where reference is made to C_{3-12} cycloalkyl, it is meant that the cycloalkyl group may contain any number of carbon atoms between 3 and 12 (*e.g.* C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}).

The term "aryl" refers to an aromatic group, such as phenyl or naphthyl. Where reference is made to C_{5-12} aryl, it is meant that the aryl group may contain any number of carbon atoms between 5 and 12 (*e.g.* C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}).

The term "C_{5-12} aryl-C_{1-6} alkyl" refers to groups such as benzyl, phenylethyl and naphthylmethyl.

Nitrogen protecting groups include silyl groups (such as TMS, TES, TBS, TIPS), acyl derivatives (such as phthalimides, trifluoroacetamides, methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl (Boc), benzyloxycarbonyl (Z or Cbz), 9-fluorenylmethoxycarbonyl (Fmoc), 2-(trimethylsilyl)ethoxy carbonyl, 2,2,2-trichloroethoxycarbonyl (Troc)), sulfonyl derivatives (such as β-trimethylsilylethanesulfonyl (SES)), sulfenyl derivatives, C_{1-12} alkyl, benzyl, benzhydryl, trityl, 9-phenylfluorenyl, *etc.* A preferred nitrogen protecting group is Fmoc.

In general, the invention does not encompass the various NMB1870 sequences specifically disclosed in references 3, 5, 6 and 7, although these NMB1870 sequences may be used according to the invention *e.g.* for the construction of chimeric sequences, *etc.*

BRIEF DESCRIPTION OF DRAWINGS

Figures 1 to 6 show 3D models of NMB1870. Figure 7 shows surface loop transfer for NMB1870.

Figure 8 shows 12mer PepScan epitope mapping of a family I NMB1870 protein. The 3 panels from top to bottom are the results using antisera generated against NMB1870 families I, II and III. Results are in arbitrary dye units.

Figure 9 shows a western blot of fragments of NMB1870, stained using polyclonal serum.

Figure 10 shows FACS analysis using antisera raised against different NMB1870 fragments.

Figure 11 shows a western blot of strains in NMB1870 family I, II or III, stained either with monoclonal antibody mAb502 (left blot) or with a polyclonal anti-NMB1870 serum (right blot).

Figure 12 shows a western blot of fragments of NMB1870, stained using mAb502.

Figure 13 shows a dot blot of fragments of NMB1870, stained using mAb502. Domains A to C were tested individually. A domain B-C fragment was also tested, as was a mixture of domains B & C.

Figures 14 and 15 show FACS analysis of bacteria. The three rows were stained with different antibodies: top = mAb502; middle = polyclonal serum; bottom = monoclonal antibody against capsular saccharide (positive control, SEAM3). The three columns in Figure 14 are all family I strains: MC58, M2934 and BZ83. The three columns in Figure 15 do not express family I NMB1870: ? NMB1870 isogenic knockout of strain MC58; family II strain 961-5945; family III strain M1239.

Figure 16 shows hydrophilicity and secondary structure analyses of the $NMB1870_{MC58}$ sequence (SEQ ID NO: 1) from residues 120 to 274.

MODES FOR CARRYING OUT THE INVENTION

Epitope mapping

12mer and 10mer fragments of $NMB1870_{MC58}$ were used for 'PepScan' epitope mapping. The fragments were immobilised on a cellulose membrane and reacted with antisera raised against one strain from each of the three NMB1870 families: (I) MC58; (II) 2996; and (III) M1239. The results of the 12mer analysis are shown in Figure 8.

The region including approximately the first 110 amino acids of NMB1870 contains linear epitopes that are common to the three families. Residues 120-183 includes family-specific epitopes. No positive peptides were seen further downstream, suggesting either that these sequences are buried in the protein's 3D structure and so could not elicit any antibodies in the serum, or that the epitopes here are discontinuous and are not seen in the 'PepScan' analysis. The following alignment shows the regions of the $NMB1870_{MC58}$ sequence (SEQ ID NO: 1) which reacted with each antiserum:

```
(1) Anti-MC58    MNRTAFCCLSLTTALILTACSSGGGGVAADIGAGLADALTAPLDHKDKGL
(2) Anti-2996    MNRTAFCCLSLTTALILTACSSGGGGVAADIGAGLADALTAPLDHKDKGL
(3) Anti-M1239   MNRTAFCCLSLTTALILTACSSGGGGVAADIGAGLADALTAPLDHKDKGL
```

(1) Anti-MC58 **QSLTLDQSVR**KNEKLKLAAQGAEKTYGNGDSLNTGKLKND**KVSRFDFIRQ**
(2) Anti-2996 **QSLTLDQSVRKNEK**LK**LAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQ**
(3) Anti-M1239 **QSLTLDQS**VRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSR**FDFIRQ**

(1) Anti-MC58 **IEVDGQLITLES**GEFQVYKQSH**SALTAFQTEQIQDSEHS**GKM**VAKRQFRI**
(2) Anti-2996 **IEVDGQLITL**ESGEFQVYKQSHSALTAFQTEQIQDSEHSGKMVAKRQFRI
(3) Anti-M1239 **IEVDGQLI**TLESGEFQVYKQSHSALTAFQTEQIQDSEHSGKMVAKRQFRI

(1) Anti-MC58 **GDIAGEHTSFDKLPEGGRATYRGTAFGSDDAG**GKLTYTIDFAAKQGNGKI
(2) Anti-2996 GDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFAAKQGNGKI
(3) Anti-M1239 GDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFAAKQGNGKI

(1) Anti-MC58 EHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKA
(2) Anti-2996 EHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKA
(3) Anti-M1239 EHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKA

(1) Anti-MC58 QEVAGSAEVKTVNGIRHIGLAAKQ
(2) Anti-2996 QEVAGSAEVKTVNGIRHIGLAAKQ
(3) Anti-M1239 QEVAGSAEVKTVNGIRHIGLAAKQ

The common epitopes for all three families are thus DKGLQSLTLDQSVR (SEQ ID NO: 21) and FDFIRQIEVDGQLI (SEQ ID NO: 22).

Based on the epitope mapping results, the NMB1870 sequence was split into three notional domains:

(A) Amino acids 1-119 (SEQ ID NO: 4)
MNRTAFCCLSLTTALILTACSSGGGGVAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKL
KLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYK

(B) Amino acids 120-184 (SEQ ID NO: 5)
QSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGG

(C) Amino acids 185-274 (SEQ ID NO: 6)
KLTYTIDFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGG
KAQEVAGSAEVKTVNGIRHIGLAAKQ

These domains were expressed individually as proteins comprising domains A, B or C (SEQ ID NOS: 4 to 6), and together as proteins comprising A-B (SEQ ID NO: 23) or B-C (SEQ ID NO: 24).

The following oligonucleotide primers were using during the construction of these proteins, and introduce *Nde*I & *Xho*I restriction sites:

Protein	**Primer SEQ ID NOS** (Fwd & Rev)
(A)	25 & 26
(B)	27 & 28
(C)	29 & 30
(A)(B)	31 & 32
(B)(C)	33 & 34

A western blot of domains A (truncated to start with VAA...), B and C, using polyclonal anti-NMB1870$_{MC58}$ as the label, are shown in Figure 9. Fusions AB and BC were also included. The final lane of the blot contains the full-length protein.

Antisera against each of domains A, B and C were able to bind to whole cells in FACS analysis (Figure 10). The fluorescence shift was strongest with domains A and C, suggesting that they may be more immunoaccessible. None of the antisera could recognise a ? NMB1870 knockout strain.

Sera were raised in mice against the proteins (and against control proteins) using either CFA or an aluminium hydroxide (AH) as adjuvant, and SBA results against three different meningococcal strains were as follows:

	MC58 FAMILY I B:15:P1.7,16b (ET5)		961-5945 FAMILY II B:2b:P1.21,16 (A4)		M1239 FAMILY III B:14:P1.23,14 (lin.3)	
Protein	**CFA**	**AH**	**CFA**	**AH**	**CFA**	**AH**
A	<4	16	<4	<4	<4	<4
B	<4	<4	<4	<4	<4	256
C	<4	512	<4	<4	<4	<4
AB	<4	<4	256	256	<4	<4
BC	32768	8192	<4	<4	<4	<4
SEQ ID NO: 1	524288	16384	2048	<8	<4	<4
SEQ ID NO: 2	<4	<4	16384	2048	<4	128
SEQ ID NO: 3	<4	<4	2048	1024	16384	4096

Within SEQ ID NO: 1 (MC58), therefore, the most important bactericidal epitopes require the presence of both domains B and C (domain BC). This suggests that the protein may include discontinuous epitopes made of sequences from both of these two domains [*cf.* ref. 237].

Moreover, monoclonal antibodies Jar1, Jar3, and Jar4, which are capable of passively protecting rats against meningococcal infection, recognise the BC domain but do not recognise domain B or C alone. Similarly, Jar5 recognises the AB domain, but not domain A or B alone.

Further details of this work can be found in reference 238.

Chimeric protein – B_{M1239}-C_{MC58}

To investigate the high bactericidal activity induced by the BC domain, a hybrid BC domain was constructed from a family III domain B (M1239 strain) and a family I domain C (MC58 strain). The B domains of families I and III show 43.8% identity.

SEQ ID NO: 1 is the full-length family I NMB1870 sequence from serogroup B strain MC58. This sequence was split into three domains: (A) aa 1-119; (B) aa 120-183; (C) aa 184-274.

SEQ ID NO: 3 is the full length family III NMB1870 sequence from serogroup B strain M1239. This sequence was also split into three domains: (A) aa 1-127; (B) aa 128-190; (C) aa 191-286.

DNA fragments coding for the B domain of M1239 and the C domain of MC58 were amplified by PCR using as template chromosomal DNA from the specific strains and the following primers:

		Sequences (SEQ ID NO:)	Restriction site
$B_{(m1239)}$	Fwd	CGCGGATCCCATATG-CAGAACCACTCCGCCGT (35)	NdeI
	Rev	GCCCAAGCTT-GCCATTCGGGTCGTCGG (36)	HindIII
$C_{(mc58)}$ - His	Fwd	GCCCAAGCTT-AAACTGACCTACACCATAGA (37)	HindIII
	Rev	CCCGCTCGAG-TTGCTTGGCGGCAAGGC (38)	XhoI

The amplified fragments were cloned sequentially in pET 21b+ as NdeI/HindIII and HindIII/XhoI fragments. The final $B_{M1239}C_{MC58}$ sequence was SEQ ID NO: 43, with the KL sequence at the start of domain C_{MC58} being contributed by a HindIII restriction site.

The protein was expressed as a C-terminal His-tag fusion protein, purified and used to immunize mice, with either AH of FCA as adjuvant. Sera were analysed for bactericidal activity against the strains representative of the three families of NMB1870. Titers were as follows:

				Meningococcal strains		
			Adjuvant	MC58 Fam I	M1239 Fam III	961-5945 Fam II
B(M1239)-C(MC58)- His			FCA	64	<4	4
B(M1239)-C(MC58)- His			AH	128	<4	16
B-C(MC58)- His			FCA	32768	<4	-
B-C(MC58)- His			AH	8192	<4	-

The titers induced by the B(1239)-C(MC58) chimera were lower against MC58 than that induced by a B(MC58)-C(MC58) control, and were negative against the M1239 strain. These results demonstrate that, in B-C chimeras, the sequence of the B domain is important for inducing bactericidal antibodies, and that domains B and C should ideally not be separated.

A monoclonal IgG2a antibody (mAb502) that recognises an epitope present only on family I proteins (Figure 11) did not recognise any of the individual domains A, B and C in western blot (Figure 12), but did recognise a domain B-C fragment. These results were also seen in a dot blot of individual domains A, B and C, or a domain B-C fragment (Figure 13). In addition, however, the antibody recognised a mixture of separate domains B and C, suggesting that the epitope recognised by the antibody can be reconstituted *in vitro* and is formed by amino acid residues on both domains. The monoclonal antibody did not recognise any of the PepScan fragments.

The juxtaposition of domains B and C may thus form a conformational epitope.

Chimeric protein – BC_{2996}-BC_{M1239}

SEQ ID NO: 2 is the full-length family II NMB1870 sequence from serogroup B strain 2996. This sequence was split into three domains: (A) aa 1-119; (B) aa 120-182; (C) aa 183-273.

Domains B and C from strain 2996 were joined to domains B and C from strain M1239, via a glycine-rich linker (SEQ ID NO: 17; GS from a *Bam*HI restriction site; SEQ ID NO: 18 as a poly-glycine linker) to make a $BC_{II}BC_{III}$ chimera (SEQ ID NO: 44). DNA encoding the domains was

amplified by PCR using as template chromosomal DNA from the specific strains, using the following primers:

		Sequences (SEQ ID NO)	Restriction site
$BC_{(2996)}$	Fwd	CGCGGATCCCATATG-CAGGACCACTCCGCCG (39)	NdeI
	Rev	CGCGGATCC-CTGTTTGCCGGCGATGCC (40)	BamHI
$BC_{(M1239)}$-His	Fwd	CGCGGATCC-**GGGGGGGGGGGG**CAGAACCACTCCGCCGT (41)	BamHI
	Rev	CCCAAGCTT-CTGTTTGCCGGCGATGCC (42)	HindIII

The sequences were cloned sequentially in pET 21b+ as Nde I/Bam HI and Bam HI/Xho I fragments. The protein was expressed as C-terminal His-tag fusion protein, purified and used to immunize mice.

Briefly, groups of 10 female CD1 mice were immunized intraperitoneally with 20μg of the protein using an aluminum or Freund's adjuvant. The protein was administered either alone, or in combination with the "three protein" combination disclosed on page 33 of ref. 239. Three immunizations were administered, and sera were taken two weeks after the third one and were analysed for bactericidal activity against the strains representative of the three NMB1870 families. Titers against three example strains for each NMB1870 family were as follows:

Serum Tested against strain	ET	COUNTRY	TYPING	NMB1870 family	NadA	BC_{2996}-BC_{M1239}		BC_{2996}-BC_{M1239} plus Triple vaccine	
						AH	FCA	AH	FCA
394/98	lin3	NZ	B:4:P1.4	1	-	<4	<4	2048	8192
44/76	ET5	NO	B:15:P1.7,16	1	-	8	8	65536	>524288
MC58	ET5	UK	B:15:P1.7,16b	1	+	<4	4	16384	512
961-5945	A4	AUS	B:2b:P1.21,16	2	+	512	8192	2048	32768
NGH38	other	NO	B:NT:P1.3	2	-	256	1024	16384	16384
M2552	other	USA	B:NT:NT	2	-	128	128	512	4096
M1239	lin3	USA	B:14:P1.23,14	3	-	256	1024	1024	8192
M3369	other	USA	B:10:P1.19,15	3	-	2048	>8192	512	2048
M01-0240988	other	UK	B:1:P1.22,14	3	+	1024	4096	4096	>8192

Thus the fusion BC domains from families II and III gives good anti-II and anti-III activity but, as expected, does not offer significant anti-I activity. The fused BC domains give lower SBA titers than a fusion of full-length (? G) NMB1870 sequences.

Chimeric protein – BC_{MC58}–BC_{M1239}–BC_{2996}

Using a similar approach, a chimera of BC domains for all three NMB1870 families was constructed. The following primers were used for amplifying domains:

		Sequences (SEQ ID NO)	Restriction site
$BC_{(mc58)}$	Fwd	CGCGGATCCCATATG-CAAAGCCATTCCGCCTTAA (46)	NdeI
	Rev	CGCGGATCC-TTGCTTGGCGGCAAGGC (47)	BamHI
$BC_{(M1239)}$	Fwd	CGCGGATCC-**GGGGGGGGGGGG**CAGAACCACTCCGCCGT (48)	BamHI
	Rev	CCCAAGCTT-CTGTTTGCCGGCGATGCC (49)	HindIII
$BC_{(2996)}$-His	Fwd	CGCGGATCC-**GGGGGGGGGGGG**-CAGGACCACTCCGCCG (50)	HindIII
	Rev	CCCGCTCGAG-CTGTTTGCCGGCGATGCC (51)	XhoI

The final sequence has SEQ ID NO: 52, with the family I sequence being joined to the family III sequence by SEQ ID NO: 17 (a BamHI restriction site, then the poly-Gly linker), and the family III sequence being joined to the family II sequence by SEQ ID NO: 45 (a HindIII restriction site, then the poly-Gly linker).

A further chimera was produced, with the BC domains in the order I-II-III rather than I-III-II. This chimera was tested in bactericidal assay, alongside a chimera of the BC domains of a family II strain fused to a family III strain, and the SEAM-3 positive control. Results were as follows:

	Strain	BC_{II}–BC_{III}	BC_{I}–BC_{II}–BC_{III}	SEAM-3
I	394/98	<4	<16	16384
	44/76	8	65536	16384
	CU385	<16	>8192	16384
	MC58	4	131072	16384
II	NGH38	1024	512	32768
	C11	<16	512	8192
	2996	<32	512	32768
	5/99	<16	<16	8192
	D8221	256	512	2048
	M2552	128	1024	4196
	M4458	<16	<16	4196
	M6208	512	2048	8192
	M5258	<16	<16	4096
	961-5945	8192	16384	8192
	M4287	<16	256	8192
	M01-240013	<16	<16	4096
	B3937	512	8192	8192
III	M1239	1024	4096	2048
	M3369	>8192	32768	8192
	M01-0240988	4096	4096	4096

In all cases but one (NGH38), therefore, the triple chimera gave better results than the double chimera. This finding was not surprising for family I strains, as the double chimera did not include a NMB1870 sequence for this family. Even for families II and III, however, results were improved.

Significantly, the number of family II strains where titres were ≥128 was 9/13 with the triple chimera, against only 6/13 for the double chimera.

Monoclonal antibody 502

To select anti-NMB1870 monoclonal antibodies with bactericidal activity, CD1 mice were immunised with family I NMB1870$_{MC58}$. Polyclonal sera from individual mice were evaluated for antibody binding by ELISA on the purified protein and on whole MC58 cells, and for complement mediated bactericidal activity against MC58. On the basis of these results the spleen of a high responder mouse was selected for the fusion with myeloma cells. Several hybridoma cell lines producing antibodies were isolated and selected by positive ELISA against the purified protein or against MC58 whole bacterial cells. MAb502, an IgG2a isotype monoclonal antibody bactericidal against MC58 strain, was selected for further studies. The antibody recognised the purified protein by ELISA and was positive in FACS analysis on strain MC58.

Western blot analysis against NMB1870 from each of the three variants confirmed that mAb502 recognises an epitope present only in family I sequences. In contrast, polyclonal serum recognised all three variants. Monoclonal mAb502 was used in FACS analysis against a number of family I strains. In each case the antibody recognised cell surface antigens (Figure 14, top row). The fluorescence shift was not as great as when using anti-NMB1870 polyclonal (middle row) or using an anti-capsule monoclonal SEAM3 (bottom row), but the binding was specific. In contrast, mAb502 did not recognise a ? NMB1870 knockout strain (Figure 15, left column) and did not recognise family II or family III strains (middle and right columns). The anti-capsule positive control recognised all of the unrecognised strains (Figure 15, bottom row).

Chimeric protein – NMB1870$_{MC58}$–NMB1870$_{M1239}$–NMB1870$_{2996}$

As an alternative approach to providing a single polypeptide containing all the three NMB1870 families, full-length proteins (except for slight N-terminus truncation, up to and including the native poly-Gly sequences *i.e.* ? G proteins) were fused to make a triple chimeric sequence SEQ ID NO: 53. The family I sequence is joined to the family III sequence by SEQ ID NO: 19 (a BamHI restriction site, then a gonococcal linker, SEQ ID NO: 20), and the family III sequence being joined to the family II sequence by SEQ ID NO: 54 (a HindIII restriction site, then the gonococcal linker). The protein was expressed as a C-terminal His-tag fusion after amplification using the following primers:

		Sequences (SEQ ID NO)	**Restriction site**
$\Delta G_{(MC58)}$	Fwd	CGCGGATCCCATATG-GTCGCCGCCGACATCG (55)	NdeI
	Rev	CGCGGATCC-TTGCTTGGCGGCAAGGC (56)	BamHI
fu(ΔG_{mc58})- chim$\Delta G_{(m1239)}$	Fwd	CGCGGATCC- GGCCCTGATTCTGACCG (57)	BamHI
	Rev	CCCAAGCTT-CTGTTTGCCGGCGATGCC (58)	HindIII
fu(chimΔG_{m1239})- chim$\Delta G_{(2996)}$-His	Fwd	CGCGGATCC- GGCCCTGATTCTGACCG (59)	HindIII
	Rev	CCCGCTCGAG-CTGTTTGCCGGCGATGCC (60)	XhoI

The protein could be purified as a soluble product after growth at 30°C.

The purified triple chimera (3-C) protein was administered to mice, and the resulting sera were tested in the bactericidal assay, using either an aluminium hydroxide (AH) or a complete Freund's adjuvant. Sera raised against the homologous NMB1870 protein (adjuvanted with AH) were also tested:

Adjuvant	Family I strain										
	MC58	M2197	BZ133	F6124	M2937	LNP17592	NZ98/254	M4030	GB185	M6190	GB345
3-C AH	131072	4096	>8192	>8192	>8192	>8192	>8192	>8192	4096	>8192	>8192
3-C FCA	16384	2048	8192	8192	>8192	>8192	4096	>8192	1024	2048	4096
Homol AH	16384	512	1024	1024	1024	512	64	2048	32	128	512
	Family II strain										
	2996	961-5945	GB013	5/99	M986	M2671	M2552	BZ232	M0579		
3-C AH	2048	>8192		1024	<16			>8192	<16		
3-C FCA	1024	>8192		256	<16			2048	<16		
Homol AH	1024	2048	<16	<16	<16	<16	128	<16			
	Family III strain										
	GB364	M3369	M1239	NGP165	GB988						
3-C AH	>8192	>8192	>8192	>8192	>8192						
3-C FCA	>8192	>8192	>8192	4096	8192						
Homol AH	1024	4096	16384	<16	2048						

In almost all cases, therefore, the triple chimeric protein gave a better bactericidal serum than the individual homologous proteins. The triple chimera thus offers two advantages: (1) coverage of all NMB1870 families in a single protein; and (2) enhanced bactericidal response relative to a single homologous NMB1870 protein.

Comparison of immunogenicity of domains

The A, B and C domains of the MC58 ? G-NMB1870 sequence (family I) were prepared singly and as AB and BC fusions, all with C-terminal His-tags. They were used to immunise mice and sera were tested for bactericidal activity against a strain from each NMB1870 family. For comparison, sera raised in response to the three families' ? G-NMB1870 sequences were also tested. Proteins were adjuvanted either with an aluminium hydroxide or with FCA. Results were as follows:

	Adjuvant	MC58 B:15:P1.7,16b ET5	961-5945 B:2b:P1.21,16 A4	M1239 B:14:P1.23,14 lin.3
A	FCA	<4	<4	<4
A	AH	16	<4	<4
B	FCA	<4	<4	<4
B	AH	<4	<4	256
C	FCA	<4	<4	<4
C	AH	512	<4	<4
AB	FCA	<4	256	<4
AB	AH	<4	256	<4
BC	FCA	32768	<4	<4
BC	AH	8192	<4	<4
MC58	FCA	524288	2048	<4
MC58	AH	16384	<8	<4
2996	FCA	<4	16384	<4
2996	AH	<4	2048	128
M1239	FCA	<4	2048	16384
M1239	AH	<4	1024	4096

Thus the individual domains are not particularly effective immunogens, the AB domain is also not particularly effective, but the BC domain shows good activity.

3D model of BC domain

A prediction of super-secondary structure for the BC domain was obtained by submitting the MC58 sequence to the HMMSTR/Rosetta server [240]. The output is shown in Figure 1.

The 3D structure was subjected to a VAST search [241] to find similarity to solved protein structures and to refine loops. The VAST output (Figure 2) was:

```
NMB1870       RHAVISGSVLYNQa--EKGSYSlg----iFGGKa-----QEVA
1K32_A  311 IAFVSRGQAFIQDvsgTYVLKVpeplrirYVRRggdtkvAFIH 353
```

Using the VAST output, the 229-259 fragment (top-right of Figure 2) was further modeled and modified by introducing turns in the backbone along the dashed line of Figure 2. The resulting model was refined by energy minimization, to give the final model of Figure 3.

This model is shown with surface loops highlighted in Figure 6.

Surface epitope mapping

A multiple sequence alignment of the BC domains of NMB1870 sequences from strains recognised in western blots mAb502 revealed various sequence information. All strains that are bound by the

antibody include residue Arg223, but this residue is His in strains that are not recognised in the blots. Even so, not all of the sequences with Arg223 produce bactericidal sera, and so the total bactericidal epitope must be broader than this single residue.

The alignment identified other residues that could be involved in the formation of a specific bactericidal epitope: Phe128 (F), Ile133 (I), Asn197 (N), Gly2217 (G), Lys249 (K), Lys260 (K) and Val262 (V). All these amino acids (grey backgrounds below) are perfectly conserved among ET-5 strains (such as MC58), which are BCA-positive, but differ in the BCA-negative strains:

```
mc58                                                KQSHSALTAFQTEQIQDSEHSGKMVAK
f6124                                               KQSHSALTALQTEQVQDSEHSGKMVAK
m198172                                             KQSHSALTALQTEQVQDSEHSGKMVAK
m4030                                               KQSHSALTALQTEQVQDSEDSGKMVAK

mc58     RQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFAAKQ
f6124    RQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDASGKLTYTIDFAAKQ
m198172  RQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDASGKLTYTIDFAAKQ
m4030    RQFRIGDIAGEHTSFDKLPKGGSATYRGTAFGSDDAGGKLTYTIDFAAKQ

mc58     GNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGI
f6124    GHGKIEHLKSPELNVDLAAIDIKPDKKRHAVISGSVLYNQAEKGSYSLGI
m198172  GHGKIEHLKSPELNVDLAAIDIKPDKKRHAVISGSVLYNQAEKGSYSLGI
m4030    GHGKIEHLKSPELNVILATAYIKPDEKRHAVISGSVLYNQDEKGSYSLGI

mc58     FGGKAQEVAGSAEVKTVNGIRHIGLAAKQ
f6124    FGGQAQEVAGSAEVETANGIKHIGLAAKQ
m198172  FGGQAQEVAGSAEVETANGIKHIGLAAKQ
m4030    FGGQAQEVAGSAEVETANGIIHIGLAAKQ
```

Similar work based on aligning sequences from strains that react with a bactericidal polyclonal serum identified the following residues: Phe128, Ile133, Asn197 and Gly221. Residues Lys249, Lys260 and Val262, which were identified by the monoclonal antibody, were discarded at this stage, as these three residues were conserved in the NMB1870 sequence of a BCA-negative strain (M2197).

From the 3D model of NMB1870, Phe134 and Ile139 are seen to lie within a predicted alpha-helix, and therefore not well accessible to antibodies. On the other hand, both the Asn197 and Gly221 are contained within surface loops and are well exposed. Both Gly221 and Asn197 are spatially close to Arg223, and could thus be part of the same epitope. Looking at Gly221, BCA-negative strains often have this small and neutral amino acid substituted with a Glu or Lys, both of which are bulky and charged. This substitution could impair proper recognition and binding of antibody to this epitope.

This analysis thus reveals five key amino acids: Phe128, Ile133, Asn197, Gly221 and Arg223. When these residues are mapped to the 3D model (Figure 4), three of them cluster at the protein surface (Figure 5). Alignment with a secondary structure prediction (Figure 16) also shows that Ile133, Asn197, Gly221 and Arg223 are located in hydrophilic regions (*i.e.* in surface loops).

Other important residues could be the dipeptide AD in position 215-216, which is substituted in most BCA-negative strains by AY, and by SD in some weakly responders.

Amino acids 197, 221 and 223 were mutated as follows:

Original amino acid(s)	Asn-197	Gly-221	Arg-223	Asn-197 & Gly-221
Substitution(s)	His	Lys	His	His & Lys

Mutagenesis used the GeneTailor™ SDM system from Invitrogen. Internal primers containing codon changes were designed according to the instruction manual specifications, and were as follows:

Primers	Sequences (SEQ ID NO:)	Mutation
741(1)-N197H for	GATTTCGCCGCCAAGCAGGGA**cAC**GGCAAAATCGAA (SEQ ID NO: 66)	AAC → cAC
741(1)-N197H rev	TCCCTGCTTGGCGGCGAAATCTATGGTGTAGGT (SEQ ID NO: 67)	N → H
741(1)-G221K for	GCCGCCGATATCAAGCCGGAT**aaA**AAACGCCATGCC (SEQ ID NO: 68)	GGA → aaA
741(1)-G221K rev	ATCCGGCTTGATATCGGCGGCGGCCAGGTCGAC (SEQ ID NO: 69)	G → K
741(1)-R223H for	GATATCAAGCCGGATGGAAAA**CaC**CATGCCGTCATCAGC (SEQ ID NO: 70)	CGC → CaC
741(1)-R223H rev	TTTTCCATCCGGCTTGATATCGGCGGCGGCCAGGTC (SEQ ID NO: 71)	R → H

To generate each mutant, 100ng of the pET-? G741$_{(1)}$-His plasmid DNA were used as template in a methylation reaction, then 12.5ng of methylated plasmid were employed as substrate in a mutagenesis reaction, using the following primer pairs:

741(1)-N197H for / 741(1)-N197H rev

741(1)-G221K for / 741(1)-G221K rev

741(1)-R223H for / 741(1)-R223H rev

PCR was performed according to the GeneTailor™ Site-Directed Mutagenesis System instruction manual. After the reaction, 10µl of the product were analysed on a 1% agarose gel, then 2µl from mutagenesis reaction mixture were transformed into DH5a^{TM}-T1^{R} *E.coli* strain according to manual specifications. Positive colonies were analysed by plasmid isolation (QIAprep Spin Miniprep Kit, QIAGEN™) and sequencing. To generate the double-mutant ? G741$_{(1)}$-His-N197H-G221K, the DNA of the positive mutant ? G741(1)-His-G221K was used as substrate for the next one with the corresponding pair of primers 741$_{(1)}$-N197H for/741(1)-N197H rev.

For expression of the recombinant protein as C terminal-His-tag fusion, 1.5µl of each construct was used to transform *E.coli* BL21-DE$_3$ strain. Single recombinant colonies were inoculated into 4ml LB+Amp (100µg/ml), incubated at 37°C overnight, then diluted 1:30 in 20ml of LB+Amp (100µg/ml) in 125ml flasks, to give an OD_{600nm} between 0.1 and 0.2. The flasks were incubated at 37°C in a gyratory water bath shaker until OD_{600nm} indicated exponential growth suitable for induction of expression (0.4-0.8 OD). Protein expression was induced by addition of 1.0mM IPTG. After 3 hours incubation at 37°C the OD_{600nm} was measured and expression examined. 1.0ml of each sample was centrifuged in a microfuge, the pellet resuspended in PBS and analysed by SDS-PAGE

and Coomassie Blue staining. All the mutants were expressed as well as wild type and purified as soluble forms at 37°C.

Loop substitution

Based on the 3D model and on the epitope mapping work, surface loops from family II and family III of NMB1870 were transferred into a family I framework.

For Loop1, the amino acid sequence is 100% conserved among all family II and family III strains. In all the three families, Loop1 is flanked by two alpha helices (not conserved in sequence) that likely contribute to the correct exposure/folding of the loop. The Gly140 residue is not found in the family I M6190 sequence, and while monoclonals Jar3 and Jar5 bind to family I sequences they fail to bind to M6190, further confirming the epitopic importance of this loop. The family II sequence was chosen for insertion into the family I framework.

Loop2 corresponds to a bactericidal epitope. The only difference between families II and III at this position is an Asp/Gly substitution. Analysis of bactericidal responses among family II strains suggests a critical role for the Asp residue, so the family II sequence was chosen.

Loop3 is highly variable within families II and III, but is conserved in family I. In family II, a majority of strains has sequence PNG, but in family III the majority have AGG. Although this loop seems dispensable for protection (PNG strains are able to protect against family III AGG strains), to cover both possibilities the family I AG sequence was substituted with PN.

Asn197 in Loop4 was previously identified as an important residue to discriminate between BCA-positive and BCA-negative strains of family I, and is always conserved in ET-5 strains. This residue is substituted by His in all family II strains and in a subgroup of family III strains. In addition, His is also present in some family I strains. His was thus used in this loop, to cover families II and III, plus any family I sequences not covered by the MC58 sequence.

Loop5, Loop6 and Loop7 are the same in families II and III, but different from family I. For these three loops the family II/III sequences were used. The second residue in the loop is Gly rather than Val because family II strains that are susceptible to serum raised against the strain 2996 protein have this residue at that position.

The substitutions are shown in Figure 7, to change SEQ ID NO: 1 into SEQ ID NO: 61. Loops 1, 2 and 4 received family II sequences; loop 3 received the family III sequence; and loops 5, 6 and 7 received a sequence common to both families II and III. With 28 substitutions out of 274 (SEQ ID NO: 1) and 273 (SEQ ID NO: 61) amino acids in total, the overall identity after surface loop exchange remains at >90%.

The loops were substituted in series. Seven proteins were made in this series, with each loop of the ? GNMB1870$_{MC58}$ sequence being substituted in order. The seven substituted proteins were referred to as LP_1, LP_2, ..., LP_7. The LP_7 mutant is SEQ ID NO: 61.

The GeneTailor™ SDM system was used for this work (see above), with the following primers:

Primers	Sequences	SEQ ID NO
741(1)-LP1 for	GCCTTTCAGACCGAGCAAATA**aAcaAccCGGAcaAaatCGacAgc**ATGGTTGCGAAACGC	72
741(1)-LP1 rev	TATTTGCTCGGTCTGAAAGGCGGTTAAGGCGGA	73
741(1)-LP2 for	GGCGAACATACATCTTTTGAC**cAGCTTCCCGAcGGCaaa**AGGGCGACATATCGC	74
741(1)-LP2 rev	GTCAAAAGATGTATGTTCGCCCGCTATGTCGCC	75
741(1)-LP3 for	ACGGCGTTCGGTTCAGACGAT**cCgaaC**GGAAAACTGACCTAC	76
741(1)-LP3 rev	ATCGTCTGAACCGAACGCCGTCCCGCGATATGTCGC	77
741(1)-LP4 for	GATTTCGCCGCCAAGCAGGGA**cAC**GGCAAAATCGAA	78
741(1)-LP4 rev	TCCCTGCTTGGCGGCGAAATCTATGGTGTAGGT	79
741(1)-LP5 for	CTGGCCGCCGCCGATATCAAG**gCcGATGaAAAAaGC**CATGCCGTCATC	80
741(1)-LP5 rev	CTTGATATCGGCGGCGGCCAGGTCGACATTGAG	81
741(1)-LP6 for	ATCAGCGGTTCCGTCCTTTAC**ggCagcGaa**GAGAAAGGCAGT	82
741(1)-LP6 rev	GTAAAGGACGGAACCGCTGATGACGGCATGGCG	14
741(1)-LP7 for	GCCGGCAGCGCGGAAGTGAAA**AtCGgcgAaaaggTACaCgAa**ATCGGCCTTGCCGCC	15
741(1)-LP7 rev	TTTCACTTCCGCGCTGCCGGCAACTTCCTGGGCTTT	16

All proteins were expressed as well as the wild-type protein and could be purified as soluble products after growth at 37°C. The proteins LP_1, LP_2, ..., LP_7 were used to immunise mice, and the resulting sera were tested against various different strains of serogroup B meningococcus, including at least three from each NMB1870 family. The starting protein (LP0) was also tested. The proteins were adjuvanted either with an aluminium hydroxide (AH) adjuvant or with FCA (F). In the following table, each row (except for the bottom row) shows the SBA for a single serum, tested against nine exemplary strains. The bottom row shows the activity of a serum obtained using the wild-type antigen from the homologous NMB1870 (*i.e.*. testing anti-$NMB1870_{MC58}$ serum against MC58, *etc.*):

Protein	Adjuvant	MC58	NZ 98/254	M4030	2996	961-5945	M2552	M1239	GB364	GB988
		ET 5	Lin..3	other	A4	A4	other	Lin.3	ET37	other
		B:15:P1.7,16b	B:4:P1.4	B:17:P1.19,15	B:2b:P1.5,2	B:2b:P1.21,16	B	B:14:P1.23,14	B:2a:P1.5,2	B:1:P1.22,14
		NMB1870 family I			NMB1870 family II			NMB1870 family III		
LP0	F	16384	<16	4096	64	128	<16	<16	256*	<16
LP0	AH	8192	<16	256	<4	64	<16	<16	<16	<16
LP1	F	32768	256	8192	<4	512	128	<16	512	<16
LP1	AH	8192	64	2048	<4	1024	128	<16	256	<16
LP2	F	16384	<16	4096	256	2048	256*	<16	2048	<16
LP2	AH	4096	128	1024	128*	4096	128	<16	512	<16
LP3	F	65536	512	4096	256	4096		<16	1024	256
LP3	AH	16384	256	1024	<16	128		<16	256	64
LP4	F	65536	2048	8192	128*	2048		<16	1024	1024
LP4	AH	16384	256	256	<16	64		<16	128	128
Hom	AH	16384	64	2048	1024	2048	128	16384	1024	2048

* = serum was bacteriostatic

Progressing from LP0 (no substitutions; NMB1870; family I) to LP7 through LP1, LP2, *etc.*, sera raised against the protein become more active against strains whose NMB1870 is in family II or family III. As family I sequences are substituted with family II/III sequences then, contrary to *a priori* expectations, bactericidal responses against family I strains show an upward trend.

Thus SEQ ID NO: 61 contains surface epitopes from families II and III, to replace family I epitopes. The chimeric polypeptide can be used to raise antibodies against NMB1870 from families II and III, and can be combined with a normal family I sequence to give multi-family antigenicity. The combination may be as a mixture of two separate polypeptides, or may be in the form of a hybrid [7] *e.g.* NH_2–X_1–SEQ:61–X_2–SEQ:1–X_3-COOH, NH_2–X_1–SEQ:1–X_2–SEQ:61–X_3-COOH, *etc.*

New NMB1870 sequences

Extensive sequence information for NMB1870 is available [*e.g.* refs 3, 5, 6 and 7]. Further new NMB1870 sequences have been found.

The sequence for strain 4243 is given as SEQ ID NO: 62, starting at the N-terminal cysteine of the mature protein. The cleaved leader peptide is the same as a normal family I sequence.

A fourth family of NMB1870 has been seen in strain M.01.0240320 ('gb320'; SEQ ID NO: 63) and in strain S10026 (SEQ ID NO: 64). The sequence from m3813 (SEQ ID NO: 21 of ref. 7; SEQ ID NO: 65 herein) can also be classified into family IV.

It will be understood that the invention is described above by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO:	Description
1	NMB1870 from strain MC58 – family I
2	NMB1870 from strains 961-5945 & 2996 – family II
3	NMB1870 from strain M1239 – family III
4-6	Domains A to C from SEQ ID NO: 1
7-9	Domains A to C from SEQ ID NO: 2
10-12	Domains A to C from SEQ ID NO: 3
13	mature domain A from SEQ ID NO: 4
14-16	SDM primers
17-20	Linkers & expression sequences
21-22	Common epitopes
23	AB
24	BC
25-42	Primers
43	$B_{M1239}C_{MC58}$
44	$BC_{2996}BC_{M1239}$
45	Linker
46-51	Primers
52	BC_{MC58}–BC_{M1239}–BC_{2996}
53	$NMB1870_{MC58}$–$NMB1870_{M1239}$–$NMB1870_{2996}$
54	Sequence for expression
55-60	Primers
61	Surface loop substitution
62-65	NMB1870 sequences
66-82	SDM Primers

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CLAIMS

1. A chimeric NMB1870 protein that comprises portions of NMB1870 from different NMB1870 families.

2. A chimeric polypeptide according to claim 1, comprising: (a) a domain 'B' sequence from a first NMB1870 family; and (b) a domain 'C' sequence from a second NMB1870 family, wherein the first and second family are different from each other and are selected from family I, family II or family III of NMB1870, and wherein (i) the chimeric polypeptide does not contain a domain 'C' sequence from the first NMB1870 family and/or (ii) the chimeric polypeptide does not contain a domain 'B' sequence from the second NMB1870 family, and wherein (1) domain 'B' is the fragment of said NMB1870 which, when aligned to SEQ ID NO: 1 using a pairwise alignment algorithm, starts with the amino acid aligned to Gln-120 of SEQ ID NO: 1 and ends with the amino acid aligned to Gly-183 of SEQ ID NO: 1; and (2) domain 'C' is the fragment of said NMB1870 which, when aligned to SEQ ID NO: 1 using a pairwise alignment algorithm, starts with the amino acid aligned to Lys-184 of SEQ ID NO: 1 and ends with the amino acid aligned to Gln-274 of SEQ ID NO: 1.

3. A chimeric polypeptide according to claim 1, comprising: (a) a domain 'B' sequence from a first NMB1870 family; and (b) a domain 'C' sequence from a second NMB1870 family, wherein the first and second family are different from each other and are selected from family I, family II or family III of NMB1870, and wherein the chimeric polypeptide is less than 495 amino acids long.

4. A chimeric polypeptide according to claim 1, comprising amino acid sequence $-X_1-B-X_2-C-X_3-$, wherein: $-X_1-$ is an optional amino acid sequence; $-X_2-$ is an optional amino acid sequence; $-X_3-$ is an optional amino acid sequence; -B- is a domain B amino acid sequence from a NMB1870 sequence in a first family; and -C- is a domain C amino acid sequence from a NMB1870 sequence in a second family, wherein the first and second family are different from each other and are selected from family I, family II or family III of NMB1870.

5. A chimeric polypeptide according to claim 1, comprising: (a) a domain 'B' sequence and a domain 'C' sequence from a first family of NMB1870; and (b) a domain 'B' sequence and a domain 'C' sequence from a second family of NMB1870, wherein the first and second family are different from each other and are selected from family I, family II or family III of NMB1870, and wherein the chimeric polypeptide (i) does not contain a domain 'A' sequence from the first family and/or (ii) does not contain a domain 'A' sequence from the second family.

6. A chimeric polypeptide according to claim 1, comprising an amino acid sequence $-X_1-B_j-X_2-C_j-X_3-B_k-X_4-C_k-X_5-$, wherein: $-X_1-$ is an optional amino acid sequence; $-X_2-$ is an optional amino acid sequence; $-X_3-$ is an optional amino acid sequence; $-X_4-$ is an optional amino acid sequence; $-X_5-$ is an optional amino acid sequence; $-B_j-$ is a domain 'B' amino acid sequence from a first NMB1870 family; $-C_j-$ is a domain 'C' amino acid sequence from the first family; $-B_k-$ is a

domain 'B' amino acid sequence from a second NMB1870 family; and -C_k- is a domain 'C' amino acid sequence from the second family, wherein the first and second family are different from each other and are selected from family I, family II or family III of NMB1870.

7. A process for producing a chimeric NMB1870 amino acid sequence, comprising the steps of: (a) aligning a first NMB1870 amino acid sequence with a second NMB1870 amino acid sequence, to give a pair of aligned sequences; (b) selecting a portion of the first amino acid sequence, starting at amino acid a_1 of said first amino acid sequence and ending at amino acid b_1 of said first amino acid sequence; (c) selecting a portion of the second amino acid sequence, starting at amino acid a_2 of said second amino acid sequence and ending at amino acid b_2 of said second amino acid sequence, wherein residues a_1 & a_2 and b_1 & b_2 are aligned in the pair of aligned sequences; and (d) replacing said portion of the first amino acid sequence with said portion of the second amino acid sequence, thereby providing the chimeric NMB1870 amino acid sequence.

8. The process of claim 7, wherein the first and second sequences are different and are from different NMB1870 families.

9. The process of claim 8, wherein the first sequence is a family I NMB1870 sequence.

10. The process of any one of claims 7 to 9, wherein the selected portions are at least 3 amino acids long.

11. The process of any one of claims 7 to 10, wherein the portions are surface loop sequences.

12. A chimeric polypeptide according to claim 1, comprising a chimeric NMB1870 amino acid sequence obtainable by the process of any one of claims 7 to 11.

13. A chimeric polypeptide according to claim 1, comprising an amino acid sequence F_1-X_1-F_2, where: F_1 is a N-terminus fragment of a first NMB1870 amino acid sequence; F_2 is a C-terminus fragment of a second NMB1870 amino acid sequence; X_1 is an optional amino acid sequence; said first and second NMB1870 amino acid sequences are from different NMB1870 families; fragments F_1 and F_2 are both at least 10 amino acids in length; and fragments F_1 and F_2 have a combined length of at least 200 amino acids.

14. A chimeric polypeptide according to claim 1, comprising an amino acid sequence $(F^m\text{-}X^m)_n$, where: n is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10; each F^m is a fragment of a m^{th} NMB1870 amino acid sequence; each -X^m- is an optional amino acid sequence; each fragment F^m is at least 7 amino acids in length; and the n instances of F^m include fragments from at least two of the three NMB1870 families I, II and III.

15. The polypeptide of claim 14, wherein n is 3 and comprising amino acid sequence F^1-X^1-F^2-X^2-F^3-X^3.

16. The polypeptide of claim 14, wherein n is 5 and comprising amino acid sequence F^1-X^1-F^2-X^2-F^3-X^3-F^4-X^4-F^5-X^5.

17. A chimeric polypeptide according to claim 1, comprising at least two of: (i) a fragment of no more than 240 amino acids of a family I NMB1870 sequence, wherein the fragment comprises an epitope of said family I NMB1870 sequence; (ii) a fragment of no more than 240 amino acids of a family II NMB1870 sequence, wherein the fragment comprises an epitope of said family II NMB1870 sequence; and (iii) a fragment of no more than 240 amino acids of a family III NMB1870 sequence, wherein the fragment comprises an epitope of said family III NMB1870 sequence.

18. A chimeric polypeptide according to claim 1, comprising a modified amino acid sequence of a first family of NMB1870, wherein the modified sequence includes at least one surface loop sequence from a second family of NMB1870 in place of a surface loop sequence from the first family.

19. A chimeric polypeptide according to claim 1, comprising an amino acid sequence:

$$-B_1-L_1-B_2-L_2-B_3-L_3-B_4-L_4-B_5-L_5-B_6-L_6-B_7-L_7-B_8-$$

wherein:

(a) B_1 is amino acids 1-133 of SEQ ID NO: 1; B_2 is amino acids 142-161 of SEQ ID NO: 1; B_3 is amino acids 169-180 of SEQ ID NO: 1; B_4 is amino acids 183-196 of SEQ ID NO: 1; B_5 is amino acids 198-218 of SEQ ID NO: 1; B_6 is amino acids 224-233 of SEQ ID NO: 1; B_7 is amino acids 237-260 of SEQ ID NO: 1; and B_8 is amino acids 268-274 of SEQ ID NO: 1;

(b) L_1 is either amino acids 134-141 of SEQ ID NO: 2 or amino acids 142-149 of SEQ ID NO: 3; L_2 is either amino acids 162-167 of SEQ ID NO: 2 or amino acids 170-175 of SEQ ID NO: 3; L_3 is either amino acids 180-181 of SEQ ID NO: 2 or amino acids 188-189 of SEQ ID NO: 3; L_4 is either amino acid 196 of SEQ ID NO: 2 or amino acid 204 of SEQ ID NO: 3; L_5 is either amino acids 218-222 of SEQ ID NO: 2 or amino acids 226-230 of SEQ ID NO: 3; L_6 is either amino acids 233-235 of SEQ ID NO: 2 or amino acids 241-243 of SEQ ID NO: 3; and L_7 is either amino acids 260-266 of SEQ ID NO: 2 or amino acids 268-274 of SEQ ID NO: 3.

20. A chimeric polypeptide according to claim 1, comprising an amino acid sequence that has an overall sequence identity to SEQ ID NO: 1 of at least 80%, wherein: the sequence identity of said amino acid sequence to SEQ ID NO: 1 is more than 80% at the backbone regions of SEQ ID NO: 1; and the sequence identity of said amino acid sequence to SEQ ID NO: 1 is less than 80% at the loop regions of SEQ ID NO: 1.

21. A chimeric polypeptide according to claim 1, comprising an amino acid sequence that has an overall sequence identity to SEQ ID NO: 2 of at least 80%, wherein: the sequence identity of said amino acid sequence to SEQ ID NO: 2 is more than 80% at the backbone regions of SEQ ID NO:

2; and the sequence identity of said amino acid sequence to SEQ ID NO: 2 is less than 80% at the loop regions of SEQ ID NO: 2.

22. A chimeric polypeptide according to claim 1, comprising an amino acid sequence that has an overall sequence identity to SEQ ID NO: 3 of at least 80%, wherein: the sequence identity of said amino acid sequence to SEQ ID NO: 3 is more than 80% at the backbone regions of SEQ ID NO: 3; and the sequence identity of said amino acid sequence to SEQ ID NO: 3 is less than 80% at the loop regions of SEQ ID NO: 3.

23. A polypeptide comprising a fragment of a family I NMB1870 sequence, provided that (a) said fragment includes amino acid Arg-223 (b) said polypeptide comprises neither (i) a complete family I NMB1870 amino acid sequence nor (ii) a complete family I ? G-NMB1870 amino acid sequence.

24. A polypeptide comprising amino acid sequence $-Z^1-Arg-Z^2-$, wherein: (a) -Z1- is an amino acid sequence consisting of at least 100 amino acids; (b) $-Z^2-$ is an amino acid sequence consisting of at least 30 amino acids; (c) $-Z^1-$ has at least 75% sequence identity to the 100 amino acids located immediately upstream of amino acid Arg-223 in SEQ ID NO: 1; and (d) $-Z^2-$ has at least 75% sequence identity to the 30 amino acids located immediately downstream of amino acid Arg-223 in SEQ ID NO: 1.

25. A polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 5, 6, 7, 23, 24, 43, 44, 52, 53, 61, 62, 63, 64 and 65.

26. Nucleic acid encoding a polypeptide of any preceding claim.

27. An immunogenic composition, comprising the polypeptide of any preceding claim.

28. The composition of claim 27, further comprising an aluminium salt adjuvant.

29. The composition of claim 27 or claim 28, further comprising a meningococcal PorA protein.

30. The composition of claim 27 or claim 28, further comprising an outer membrane vesicle preparation from *N.meningitidis*.

31. The chimeric polypeptide of any preceding claim, for use as a medicament.

32. A method for raising an antibody response in a mammal, comprising administering an immunogenic composition of any one of claims 27 to 30 to the mammal.

FIG. 1

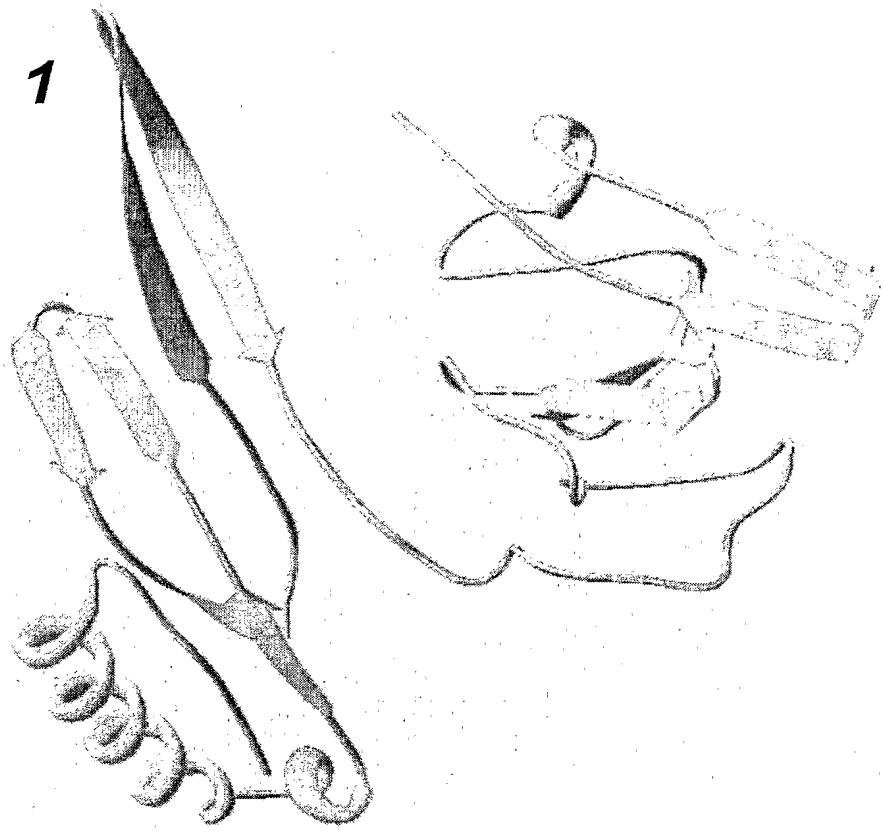


FIG. 2

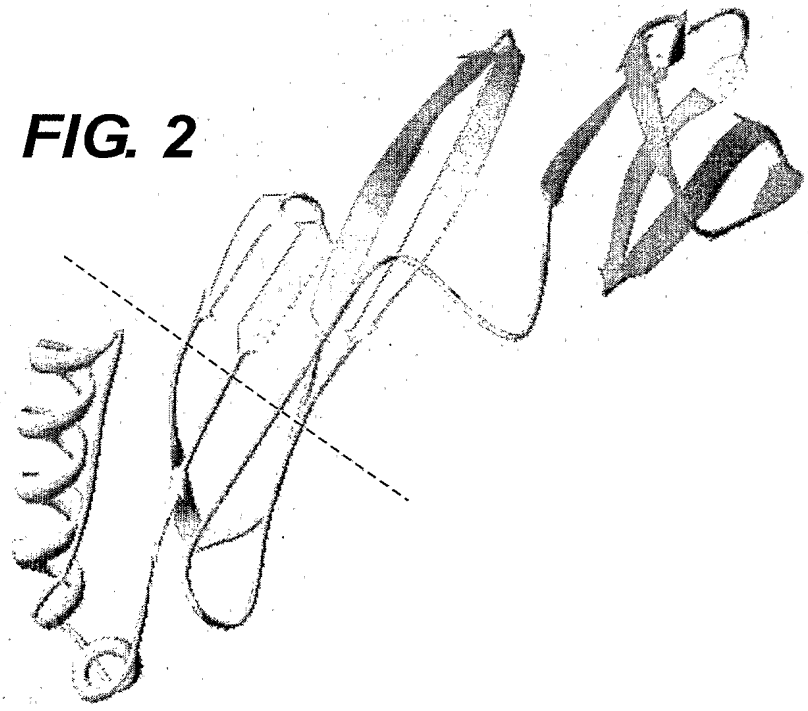


FIG. 3

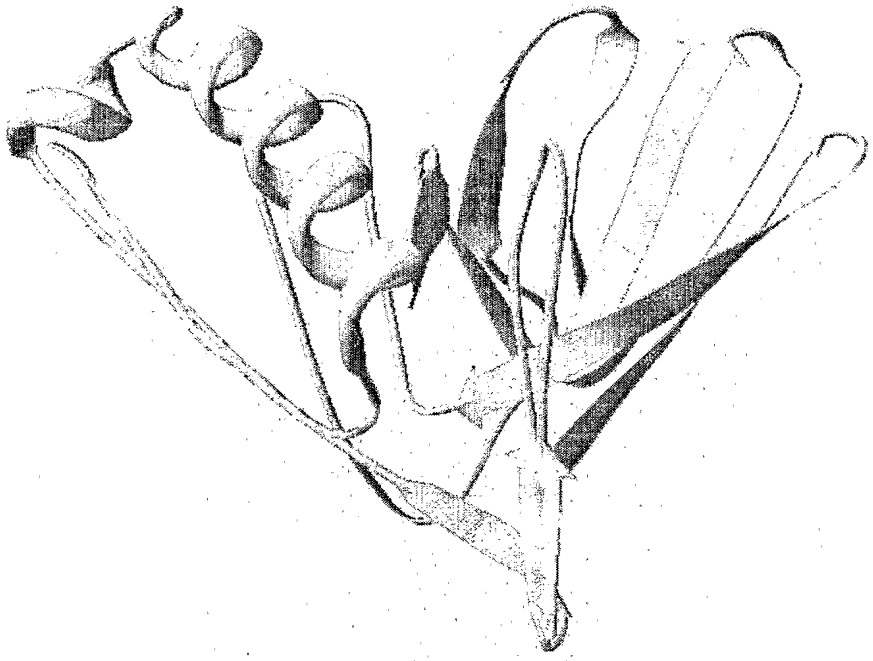


FIG. 4

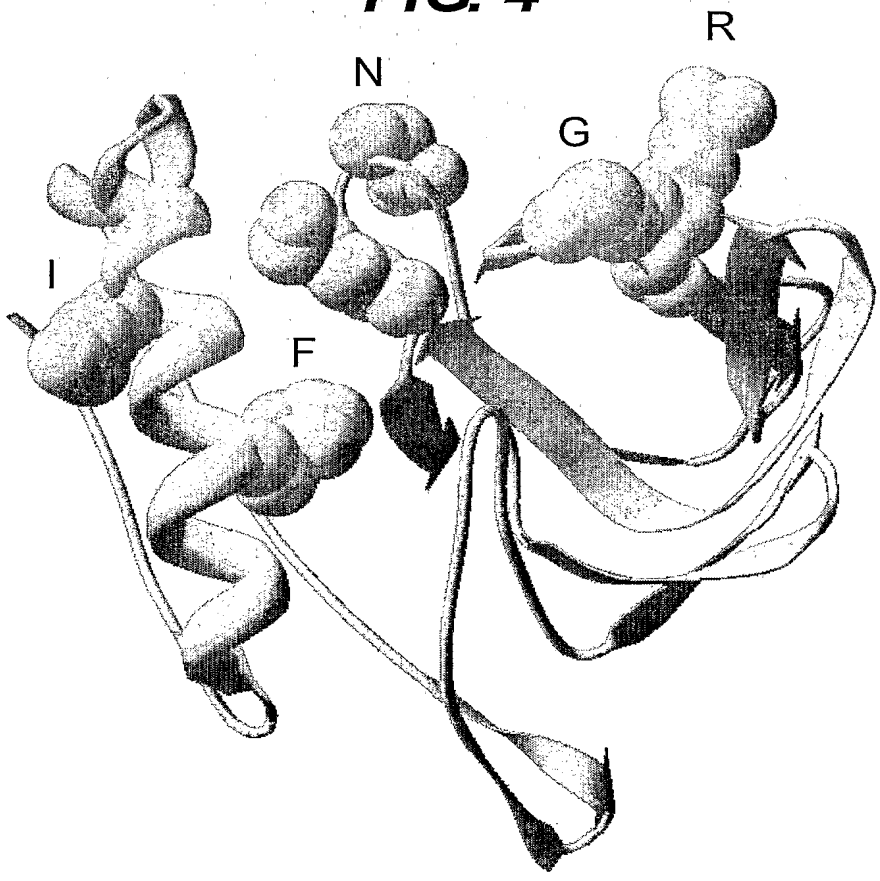


FIG. 5

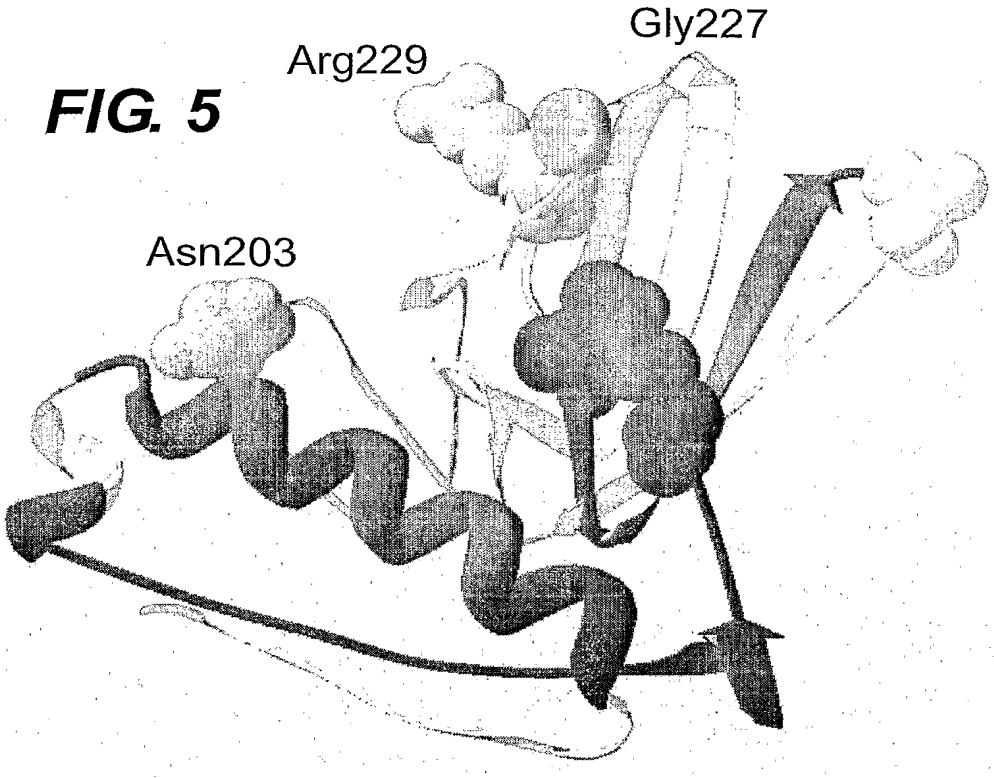


FIG. 6

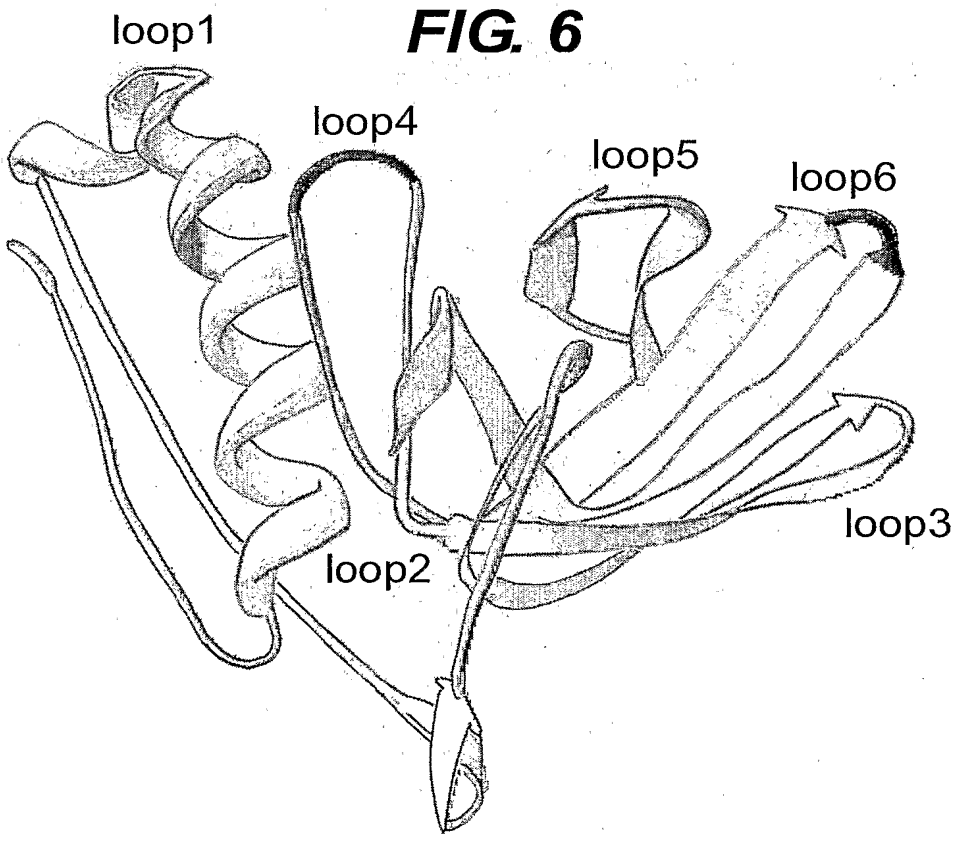


FIG. 7

MTRSKPVNRTAFCCLSLTTALILTACSSGGGGVAADIGAGLADALTAPLD
MTRSKPVNRTAFCCLSLTTALILTACSSGGGGVAADIGAGLADALTAPLD

HKDKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSR
HKDKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSR

Loop1

FDFIRQIEVDGQLITLESGEFQVYKQSHSALTAFQTEQINNPDKIDSMVA
FDFIRQIEVDGQLITLESGEFQVYKQSHSALTAFQTEQIQDSEHSGKMVA

Loop2 LOOP3

KRQFRIGDIAGEHTSFDQLPDGK-ATYRGTAFGSDDPNGKLTYTIDFAAK
KRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFAAK

Loop4 Loop5 Loop6

QGHGKIEHLKSPELNVDLAAADIKADEKSHAVISGSVLYGSEEKGSYSLG
QGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLG

Loop7

IFGGKAQEVAGSAEVKIGEKVHEIGLAAKQ
IFGGKAQEVAGSAEVKTVNGIRHIGLAAKQ

FIG. 8

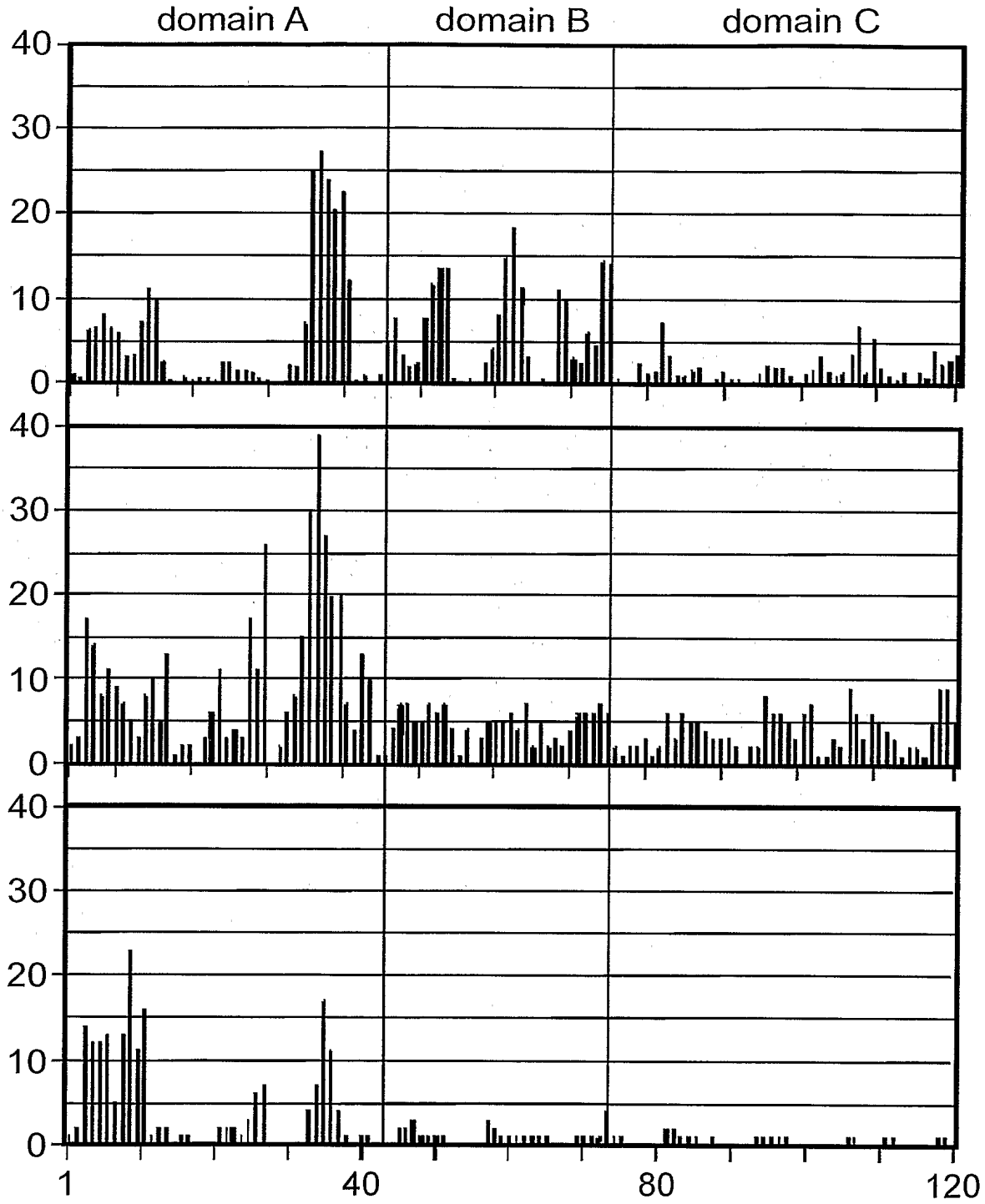

domain A
domain B
domain C
40
30
20
10
0
40
30
20
10
0
40
30
20
10
0
1
40
80
120

FIG. 9

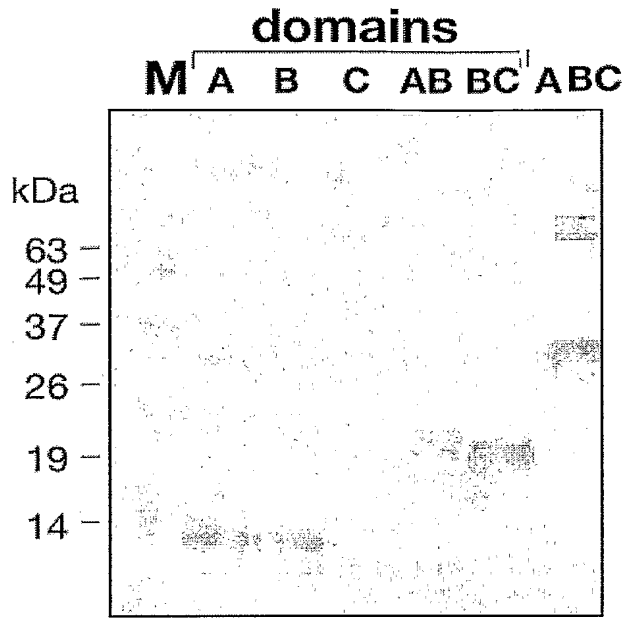

domains
M A B C AB BC ABC
kDa
63
49
37
26
19
14

FIG. 11

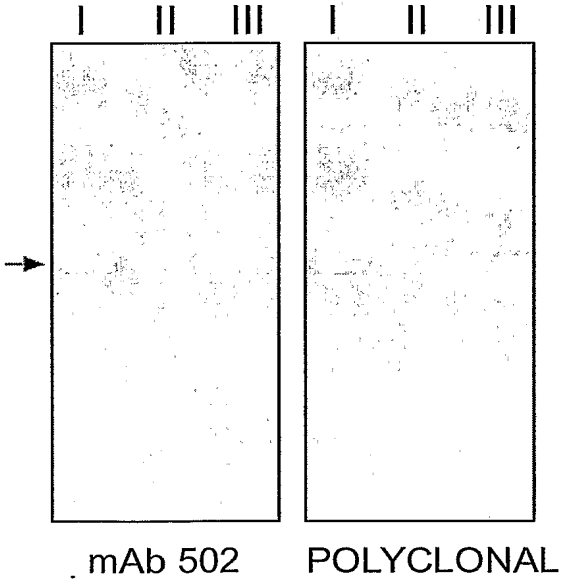

I II III I II III
mAb 502
POLYCLONAL

FIG. 10

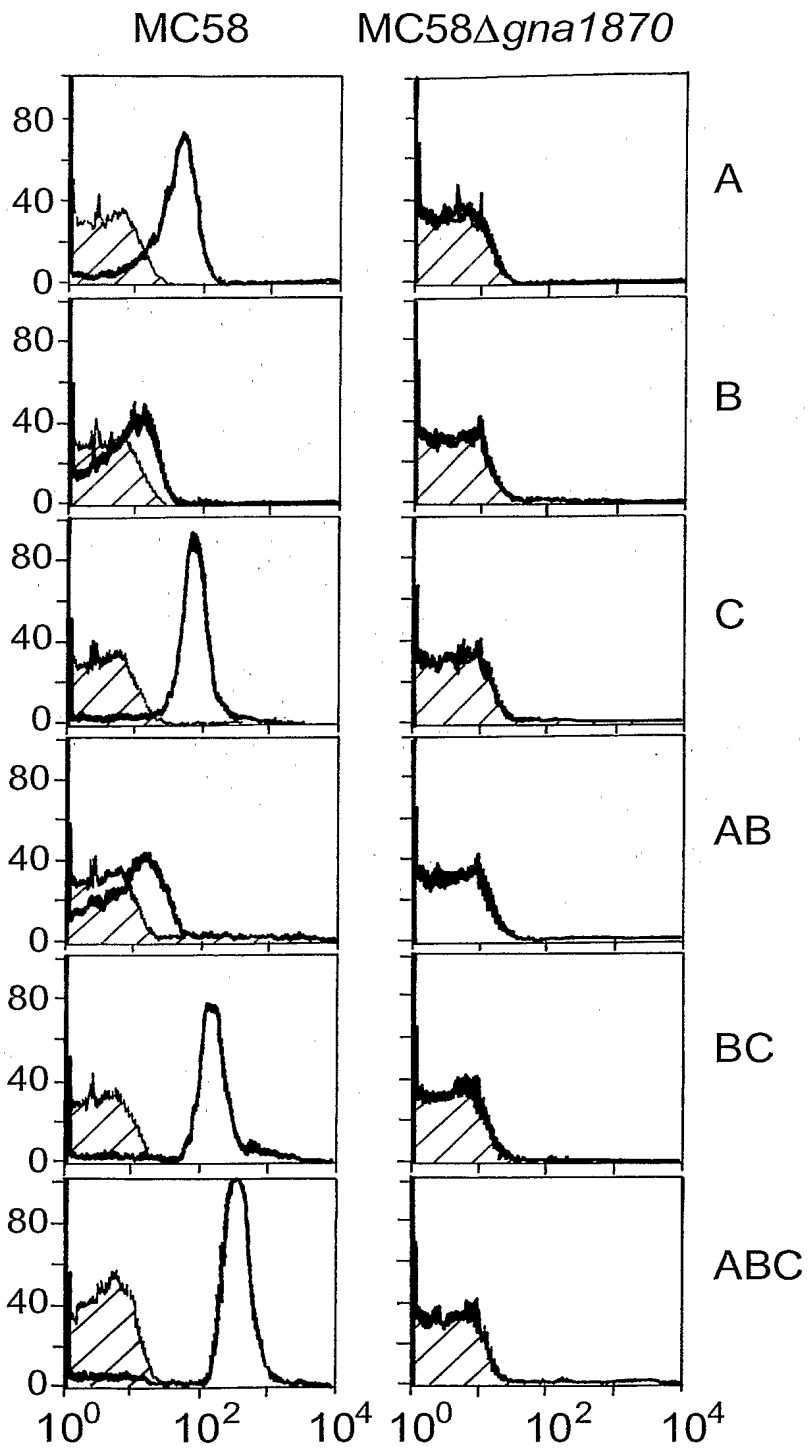


FIG. 12

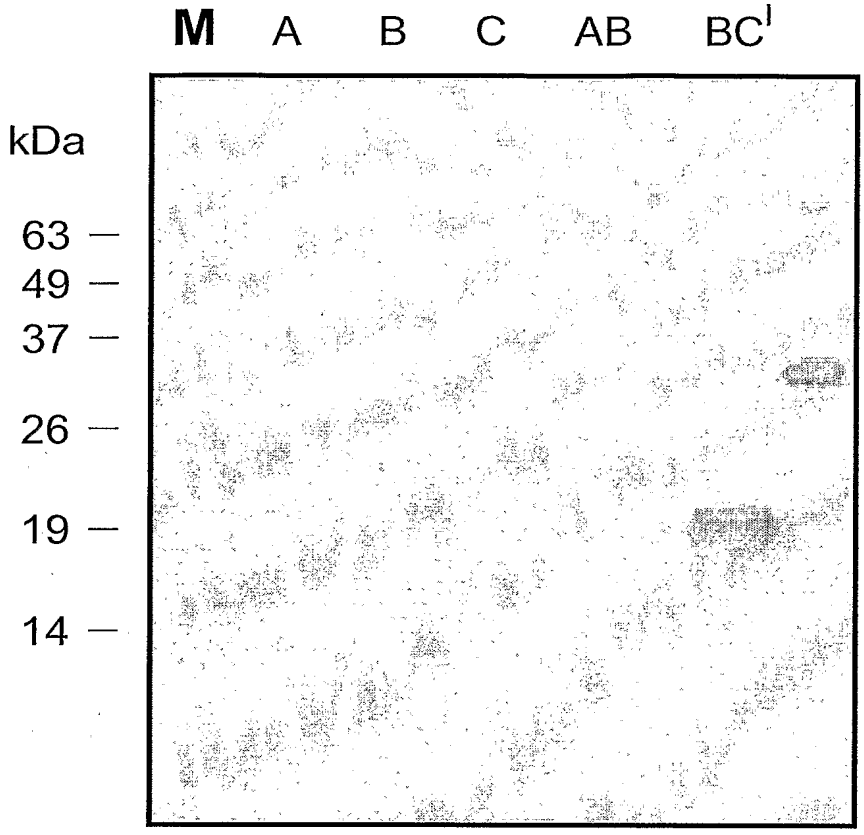


FIG. 13

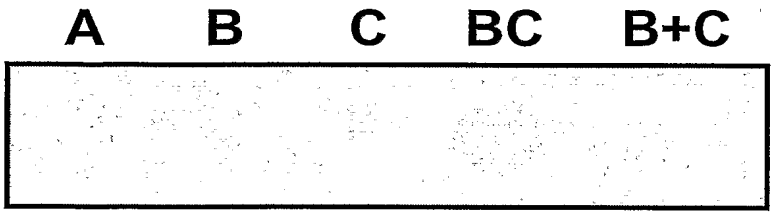


FIG. 14

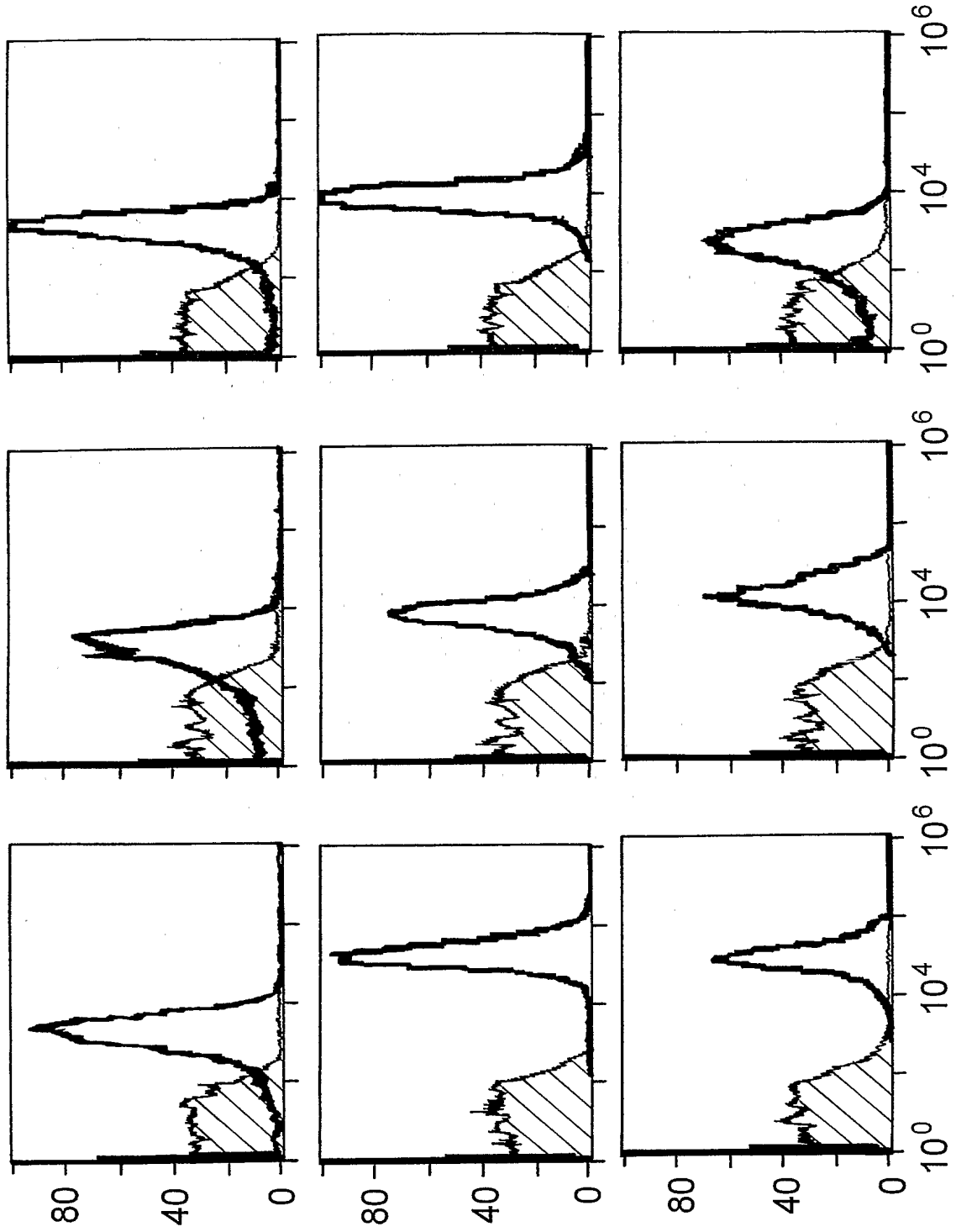
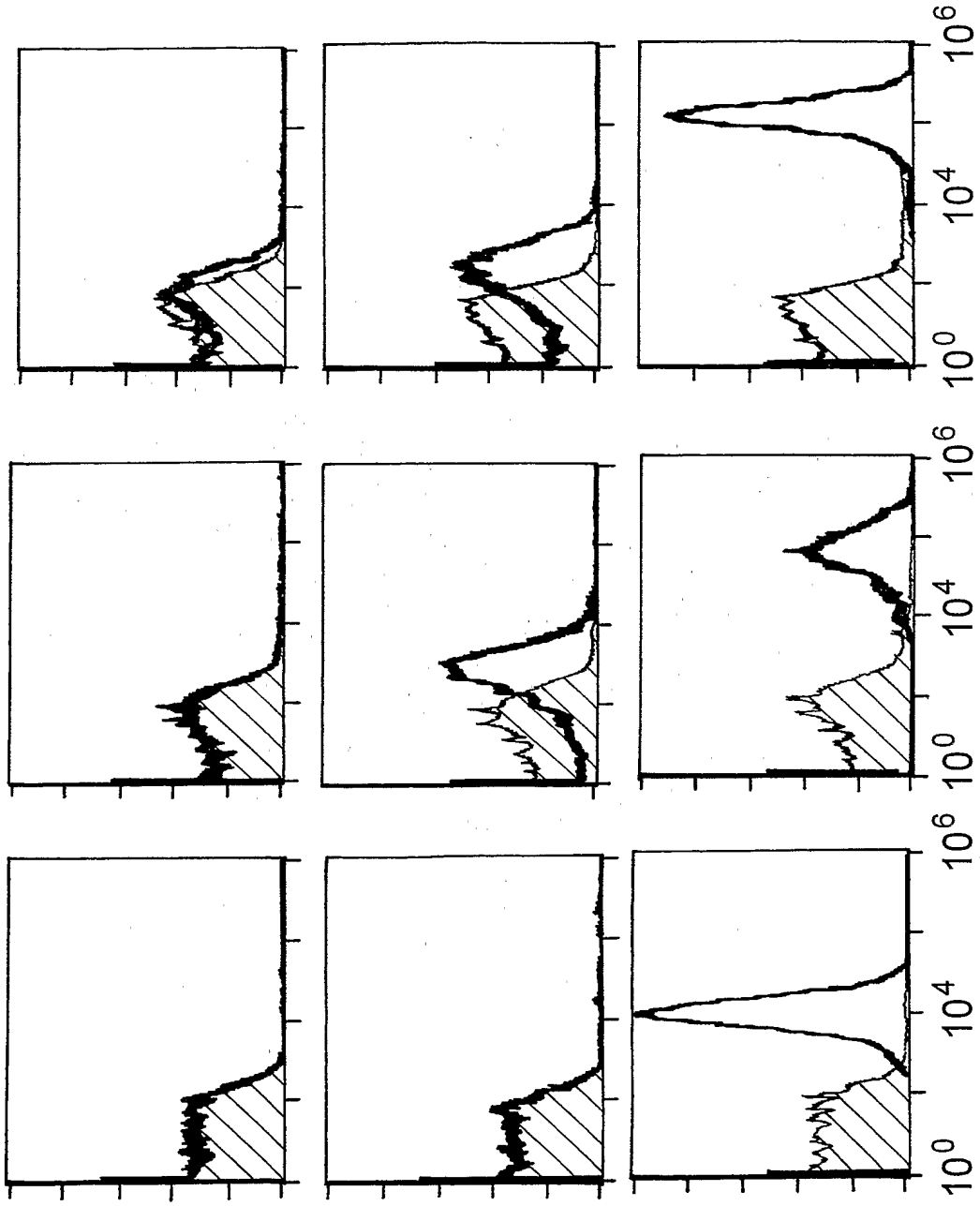


FIG. 15



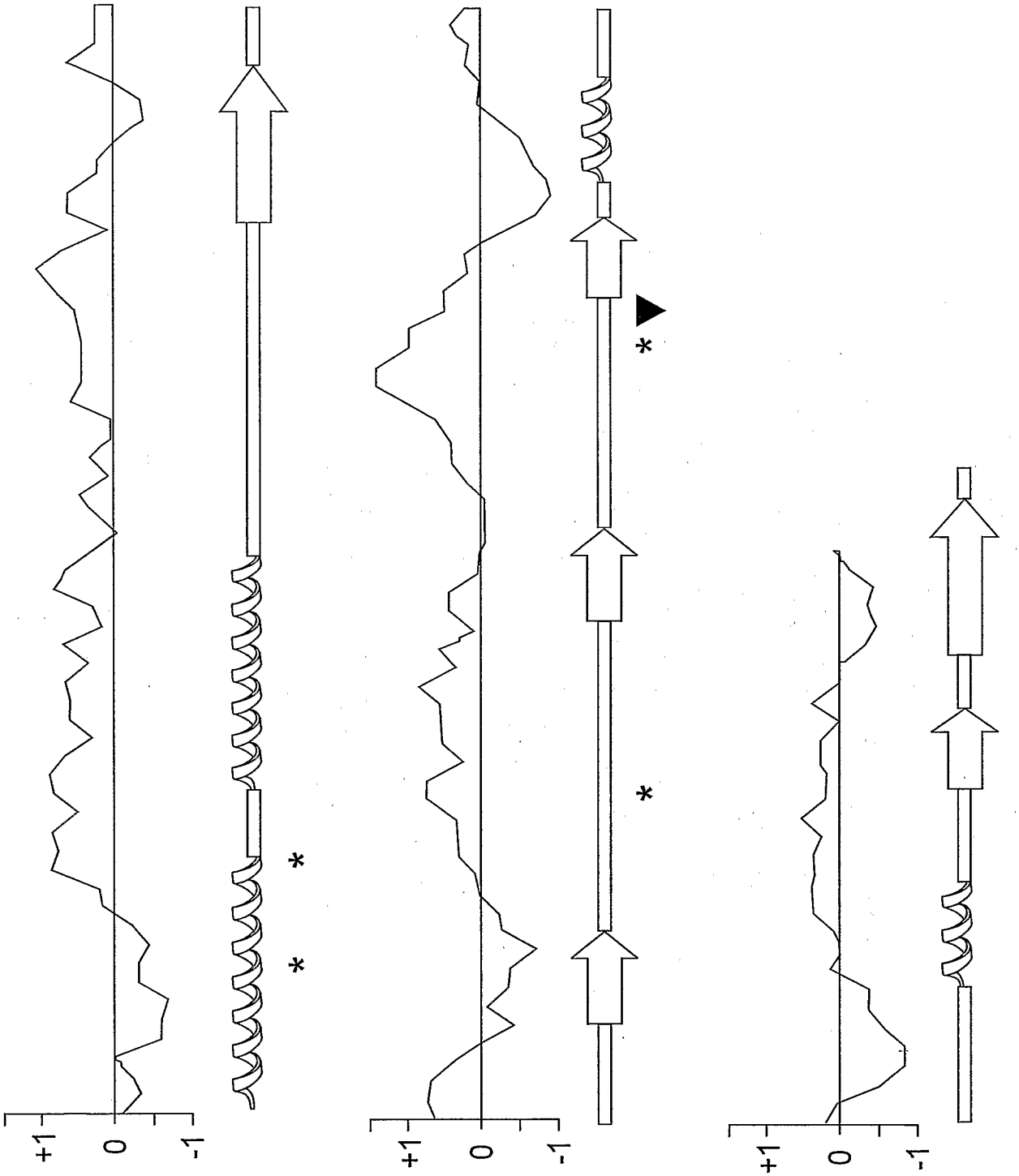


FIG. 16